Following plant community assembly in semi-natural European grasslands by analyzing environmental factors vs. history effects

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Summary

Understanding plant community assembly and succession has long preoccupied ecologists, with a plethora of different theories (stochastic, deterministic and an intermediate situation of both) put forward over time. Currently, the role of historical contingency in forming alternative vegetation states is attracting increasing attention, with priority effects caused by order of arrival of different species producing long-term and significant effects on ecosystem functioning and diversity. The role of nutrient availability in modulating the strength of priority effects is an important consideration, since significant effects of order of arrival on communities may depend strongly on how many nutrients are available in the soil.

The range and the effect size of these key drivers of assembly (historical contingency, nutrients) along temporal, spatial and resource related gradients are rarely addressed. The main underlying goal is to understand community assembly better to gain knowledge that can be applied in the restoration of species-rich grasslands, so that specific desired goals of an ecosystem can be met.

The topic of this dissertation is the investigation of priority effects in relation to soil nutrient availability over time during assembly of semi-natural European grasslands. The focus is on both community traits and species-specific traits (including intraspecific responses of traits).

The main grassland field experiment (*Manuscript 2*) showed that priority effects do play a role in community assembly of dry acidic grasslands, but the stability over time depended on the variables measured, with stable priority effects being found in relation to community aboveground biomass and plant functional group composition but not for community composition or species richness. The low nutrient availability negatively influenced the establishment of target species and the breadth of the priority effect was not as widely found in mesic grasslands, which suggested the important role of abiotic factors on community assembly.

These results supported the findings of Sutherland (1974), that the vegetation in some ways reached a stable state in terms of the plant functional group relative composition but not in terms of species richness of species composition.

Furthermore it could be shown that not the species richness of the initial seed mixture was the determining factor, but functional composition especially the plant functional group of legumes have an abiding positive effect on community traits (aboveground productivity and cover) in these grasslands, which could be also demonstrated using a new automated field measurement system (*Manuscript 1*).

In *Manuscript 3* the positive priority effect of the plant functional group of legumes on productivity found in the field, could be confirmed in a pot experiment using more nutrient-rich potting soil. In terms of productivity and functional group composition the simultaneously sown controls and the legume first treatment developed similarly. Which also underlines the importance of legumes as keystone species for facilitation or complementary effects in species-rich grasslands. In general it could be shown that the priority effect was stronger than the sowing interval or sowing density effect.

Legumes seemed to be better able to get a head-start and grow quickly, compete well and allow facilitation for neighbors (hence a combination of asymmetric competition but also functional complementarity allowing for good community and population performance when legumes arrived early). The exact mechanisms of this priority effect of legumes first, which has been since confirmed in other experiments, is not yet known, but could be related to changes in root traits and exudation and hence plant-plant interactions underground.

Manuscript 4 studied the intraspecific trait variation of one target forb species *Plantago lanceolata* across two different soil nutrient levels and in interaction with other plant species in community assembly. In mesocosms limited soil nutrients were the main factor driving variation in intraspecific traits, but it also depended on which trait one focused on (again). Intriguingly, the traits converged over time when comparing between the two soil nutrient treatments. In the field soil fertility had less influence on trait differences. This showed that the multivariate factors, which act simultaneously on the species plant trait in the field, led to a more stable trait variation, and especially legume neighborhood (again) played a role in creating a positive facilitation and complementarity effect.

The results of this thesis show us the importance of also focusing on plant functional groups, especially legumes in interaction with other groups, when investigating priority effects in community assembly. Whether one finds a priority effect or not and how stable it is may depend on which parameters one measures. In the future we need more knowledge of the mechanisms and prevalence, relevance and stability of priority effects, so that we can then hopefully steer communities in desired directions in terms of ecosystem functions (higher hay biomass, more carbon storage or other ecosystem services).

Zusammenfassung

Die Sukzession und die Entstehung von Artengemeinschaften zu verstehen, beschäftigt Ökologen seit langem. Im Laufe der Zeit wurde daraus hervorgehend eine Vielzahl von verschiedenen Theorien (stochastisch, deterministisch, eine Mischung aus beidem) aufgestellt. Gegenwärtig gewinnt die Rolle der historischen Kontingenz bei der Bildung alternativer Vegetationszustände zunehmend an Aufmerksamkeit, wobei *Priority effects*, die durch die Reihenfolge der an einem Standort ankommenden Pflanzenarten verursacht werden, langfristige und signifikante Auswirkungen auf die Diversität und die Ökosystemfunktionen haben. Die Rolle der Nährstoffverfügbarkeit ist bei der Ausprägung von *Priority effects* ein wichtiger Gesichtspunkt, da die Reihenfolge der in einer Gemeinschaft ankommenden Arten signifikant von der Bodennährstoffverfügbarkeit beeinflusst wird.

Der Einfluss dieser Schlüsselfaktoren auf die Zusammensetzung entlang eines zeitlichen, räumlichen und ressourcenbezogenen Gradienten wird selten betrachtet. Das Hauptziel ist es, ein besseres Verständnis über die Zusammensetzung von Artengemeinschaften zu erlangen, welches bei der Restoration artenreicher Grünlandgesellschaften angewendet werden kann, um bestimmte gewünschte Ziele eines Ökosystems zu erreichen.

Das Thema dieser Dissertation ist die Untersuchung von *Priority effects* über die Zeit in Bezug auf die Bodennährstoffverfügbarkeit während der Entwicklung von naturnahen europäischen Grünlandgesellschaften. Der Schwerpunkt liegt sowohl auf Gemeinschafts-merkmalen als auch auf artspezifischen Merkmalen (einschließlich intraspezifischer Reaktionen dieser). Das Hauptfreilandexperiment (*Manuskript 2*) zeigte, dass *Priority effects* tatsächlich eine Rolle bei der Entstehung der Zusammensetzung von Trockenrasen-gesellschaften spielen. Die Stabilität dieser Effekte über die Zeit hängt jedoch von den gemessenen Variablen ab. Dabei wurden stabile *Priority effects* in Bezug auf die oberirdische Biomasse der Pflanzengemeinschaft und auf die Zusammensetzung der funktionellen Gruppen gefunden, jedoch nicht in der Zusammensetzung der Gemeinschaft oder dem Artenreichtum. Die geringe Bodennährstoffverfügbarkeit wirkte sich negativ auf die Etablierung von Zielarten aus, und die Breite des *Priority effects* war nicht so groß, wie der in Fettwiesen, was auf die wichtige Rolle der abiotischen Faktoren bei der Entstehung von Artengemeinschaften schließen lässt. Diese Erkenntnisse stützen die Ergebnisse von Sutherland (1974), dass die Vegetation in mancher Hinsicht einen stabilen Zustand in Bezug auf die relative Zusammensetzung der funktionellen Pflanzengruppe erreichen kann, jedoch nicht in Bezug auf den Artenreichtum der Artenzusammensetzung.

Außerdem konnte gezeigt werden, dass nicht der Artenreichtum der ursprünglichen Samenmischung der bestimmende Faktor war, sondern die funktionelle Zusammensetzung insbesondere die der Leguminosen, wies einen anhaltend positiven Effekt auf die Gemeinschaftsmerkmale (Produktivität und Bedeckungsgrad) in diesen Grünland-gesellschaften auf, was auch mit einem neuen automatisierten Feldmesssystem nachgewiesen werden konnte (*Manuskript 1*).

In *Manuskript 3* konnte der positive *Priority effect* der Leguminosen auf die Produktivität, welche im Freiland gefunden wurde, in einem Topfversuch unter Verwendung von nährstoffreicherem Substrat bestätigt werden. In Bezug auf Produktivität und funktionelle Gruppenzusammensetzung entwickelten sich die Kontrollbehandlungen, bei denen alle Arten zur gleichen Zeit ausgesät wurden und die Behandlungen, bei denen die Leguminosen zuerst gesät wurden, ähnlich. Dies unterstreicht wieder die Bedeutung der Leguminosen als Schlüsselarten für positive Interaktionen auf andere Arten und Komplementaritätseffekte in artenreichen Grünlandgesellschaften. Im Allgemeinen konnte dieses Experiment zeigen, dass der *Priority effect* stärker war, als das Aussaatintervall oder der Effekt der Aussaatdichte.

Leguminosen scheinen besser in der Lage zu sein, einen Vorsprung zu erlangen, schnell zu wachsen, konkurrenzfähig zu sein und einen positiven Einfluss auf Nachbararten zu haben (daher scheint eine Kombination aus asymmetrischer Konkurrenz, aber auch funktionaler Komplementarität, eine gute Leistung der Gemeinschaft zu ermöglichen, wenn sich Leguminosen früh ansiedeln). Die genauen Mechanismen dieses *Priority effects*, welcher inzwischen auch in anderen Experimenten bestätigt wurde, sind bisher noch nicht bekannt. Aber sie könnten mit Veränderungen in Eigenschaften der Wurzel und der Exudation und damit mit der unterirdischen Interaktion zwischen Pflanzen zusammenhängen.

Manuskript 4 untersuchte die intraspezifische Variation der Merkmale von *Plantago lanceolata* in Abhängigkeit von zwei sich in Nährstoffverfügbarkeit unterscheidenden Böden und in Interaktion mit anderen Pflanzenarten innerhalb der Entstehung von Arten-gemeinschaften. In den Mesokosmen war die limitierende Bodennährstoffverfügbarkeit der treibende Hauptfaktor für die Variation der intraspezifischen Merkmale, aber auch hier war es davon abhängig auf welches Merkmal man sich konzentrierte. Interessanterweise näherten sich die Merkmale, im Vergleich zu den beiden Bodennährstoffbehandlungen, im Laufe der Zeit an. Im Freiland hatte die Bodennährstoffverfügbarkeit weniger Einfluss auf die Variation der Merkmale. Dies zeigte, dass die multivariaten Faktoren, die gleichzeitig auf die Merkmale der Pflanzenarten im Freiland einwirken, zu einer stabileren Variation der Merkmale führten und insbesondere die Nachbarschaft von Leguminosen auch hier wieder eine entscheidende

Rolle bei der Entstehung von positiven Interaktionen auf andere Arten und Komplementaritätseffekte spielte.

Die Ergebnisse dieser Arbeit zeigen die Wichtigkeit sich bei der Untersuchung von *Priority effects* in der Entstehung von Artengemeinschaften auch auf die funktionellen Pflanzengruppen zu konzentrieren, insbesondere die der Leguminosen im Zusammenspiel mit anderen Gruppen. Ob man *Priority effects* findet und wie stabil diese über die Zeit sind hängt von den jeweilig gemessenen Parametern ab. In Zukunft brauchen wir mehr Wissen über die Mechanismen und die Prävalenz, die Relevanz und die Stabilität der *Priority effects*, damit wir die Artengemeinschaften hoffentlich in die gewünschten Richtungen hinsichtlich der Ökosystemfunktionen (Heubiomasse, mehr Kohlenstoffspeicherung oder andere Ökosystemleistungen) steuern können.

1 Introduction

1.1 Background of the thesis

1.1.1 Community assembly

Understanding how communities assemble over time is complex and has long been a central question to community ecology (see section below) of importance for practical conservation and ecological restoration (see review Wainwright *et al.* 2018) that consider the dynamics of communities.

Ecological succession and assembly remain key topics in ecology, including the issue of what mechanisms are behind succession. In general, ecological succession and assembly are very similar processes, but assembly theory focuses more on the detailed interactions between plant species whereas succession theory focuses more on the overall changes over time after disturbances (e.g. volcanic eruptions (primary succession) or plowing in conventional agriculture (secondary succession)) (Hobbs *et al.* 2007). Irrespective of whether one uses an assembly or succession framing, knowing more about the mechanisms will be an important tool for being able to predict assembly (Temperton *et al.* 2004).

Generally, succession/assembly theories can be grouped into four categories:

1. deterministic (Clementsian, niche related, abiotic environmental factors), where communities assembled to general principles,
2. stochastic (Gleasonian), where communities assembled dependent on uncertain local (abiotic and biotic factors (interactions among and between species and their abiotic environment)) and historical effects (priority effects)
3. neutral (Hubbell 2001), where species extinction and immigration or speciation of new species controlled the number of species in a community with the assumption that all individuals of all species are ecologically equivalent (Zhou & Zhang 2008),
4. and an intermediate theory (the alternative stable states model (ASS)), that includes both deterministic (abiotic environmental factors) and stochastic components (historical factors) thus leading to alternative vegetation states (Sutherland 1974) (Belyea & Lancaster 1999, Chase 2003, Zuluaga 2015).

In the early years of ecology a central topic was whether biological communities are deterministic or stochastic assemblages (Clements 1916, Gleason 1926, Connor & Simberloff 1979). For example Gleason (1926) showed that the history of species arrival can influenced

community structure strongly via stochastic processes (Kreyling *et al.* 2011, von Gillhaussen 2015, Weidlich *et al.* 2017). In contrast Clements (1916) view was that community structure is highly deterministic towards a defined and finally stable climax community controlled by environmental conditions. It can be stated that for long the Clementsian and the Gleasonian approach stood in clear contrast, representing a more deterministic view leading to the same result (community) under certain conditions (Clementsian) and a more individualistic view resulting from stochastic processes (Gleasonian). Both concepts are justified, and both are insufficient to a certain degree, and both have been used similarly by a legacy of scholars and even schools such as the Zurich-Montpellier-School of phytosociology (Braun-Blanquet 1928), which followed a comparable holistic and deterministic approach as Clements.

Diamond (1975) famously coined the term "assembly rules" in his study of a tropical bird community with a primary focus on how biotic interactions (e.g. competition) shape local communities. Later, other authors (Roughgarden 1989, Drake 1990) recognized that communities are structured not only by biotic interactions also by abiotic constraints like the environment (Booth & Larson 1999).

In Keddy (1992) assembly rules are described as any ecological process filtering for or against specific traits (and therefore for the subset of species) from a regional species pool thus determining the composition of the local community (Götzenberger *et al.* 2012). The filtering or constraints are therefore produced by climate conditions, disturbance regime, abiotic and biotic interactions. Belyea & Lancaster (1999) and Chase (2003) described community assembly using external factors (dispersal and environmental constraints), internal dynamics between species and the history of species. A specific history of species invasion can than lead to different final community composition (multiple stable equilibria), even when the environmental conditions would be similar and all species would have access (*Manuscript 2*). Chase (2003) also highlighted that the size of the species pool, dispersal rate, disturbance rate, level of productivity, and the connectance rate within landscapes, can influence whether ecosystems develop to a single or a multiple stable equilibrium. The questions of how stable these equilibria are, or whether they are merely stable states (possibly transitory ones) have also received attention (Fukami & Nakajima 2011). To understand historical contingency in community assembly Fukami & Nakajima (2011) argued for a conceptual shift of focus from alternative stable states to alternative transient states. Soil conditions are also important drivers Conradi *et al.* (2017) show in a field experiment that soil nutrient availability is a critical environmental feature that dictates the degree to which

terrestrial plant communities are controlled by niche‐based selection versus stochastic assembly processes (see also *Manuscript 2*).

Both regional and local factors like environment and history should determine the patterns by which communities assemble, which is the main focus in this work. Fukami *et al.* (2005) and Helsen *et al.* (2012) show in grassland experiments that community assembly could be deterministic and result in distinct communities but this depends on different level trait or species identity. In these cases they found that trait convergence over time (niche related) and species identity divergence, caused by historical processes.

Hence, ecological research on community assembly, i.e. the composition of communities with a certain set of species in a certain spatio-temporal arrangement, can be seen as the study of the process and the mechanisms that are forming local communities out of a given species pool (Keddy 1992). Community assembly aims to understand the processes that determine the patterns of the number and composition of co-occurring species. Community assembly patterns show strong scale dependence (Drake 1990, Sanders *et al.* 2007), due to processes like filter effects (abiotic and biotic), species pool and microsites operating at a wide range of spatio-temporal scales. Kraft & Ackerly (2013) state: "Community assembly considers both the ecological interactions (dispersal, abiotic and biotic interactions (ecological assembly)) that shape the local communities and also the evolutionary and biogeographic processes (phylogeographic assembly) that lead to variation in the diversity and composition of the potential species pool through speciation, extinction and migration of species" (Figure 1). The latter (phylogeographic assembly) plays a subordinated role in this work.

The co-occurrence of species can be seen as a product of chance, historical patterns (land use, speciation, migration), dispersal, abiotic filters and biotic interactions (Götzenberger *et al.* 2012). All these processes can be used to make inferences about community assembly mechanisms and none will be mutually exclusive. Identifying and disentangling the different mechanisms and processes behind community assembly can help to understand how communities will behave under changing and future environmental scenarios (Götzenberger *et al.* 2012). Within this work, the main interest is in the understanding of ecological assembly. The study wants to disentangle whether changes in communities depended on deterministic and/or stochastic factors.

Plants are sessile organisms. To become a member of an actual community, their diaspores first have to arrive at the local site. The probability of arrival depends on the regional species pool (dispersal assembly). Second, individuals need to be successfully established on a respective site under the given abiotic and biotic conditions (abiotic and biotic assembly).

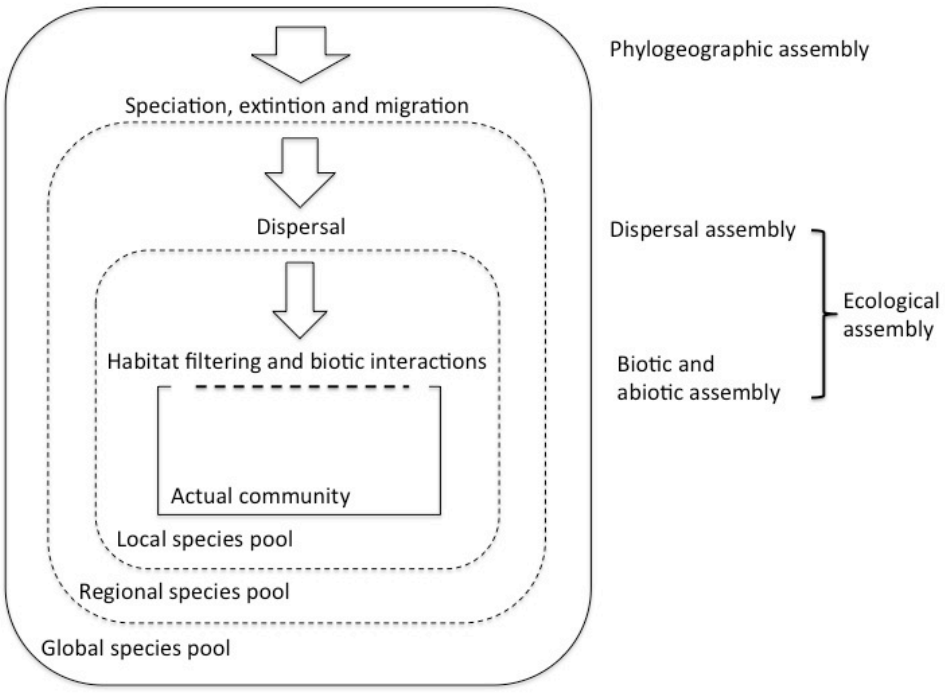

Figure 1. Schematic view of the different processes and drivers of assembly and the relative scales at which they are most influential (adapted from Götzenberger *et al*. 2012). "At any point in time there is a global species pool that defines a regional species pool through the speciation, extinction and migration of species (phylogeographic assembly). At a given local site the species pool constitutes species from the regional species pool that are able to disperse there (dispersal assembly). At the local site, habitat filtering and biotic interactions define the actual assemblage of plant species (ecological assembly)" (Götzenberger *et al.* 2012).

Different mechanisms for seed dispersal apply such as gravity, floating on water, movement by wind or transport by animals. All mechanisms include a stochastic component and can be unspecific with respect to the spatial pattern of environmental conditions or biotic structures where a particular species is most likely to germinate and successfully establish (Kraft & Ackerly 2013). The travelling distance and numbers of seeds that arrive on a site is also a stochastic component. In disturbed sites dispersal limitations may occur e.g. due to complete mortality of all living individuals in the system, as well as loss of the entire soil complex (Emery 2010, Makoto & Wilson 2019).

Filter effects

As far as the abiotic and biotic assembly is concerned, the difference between the available regional or local species pool and the actual extant community of plants found at a site will have been affected by filtering effects of abiotic and biotic filters that only "allow" certain species with particular traits or phenotypes to pass through the mesh and establish (Harper 1977 see Hobbs & Norton 2004, Nobel & Slatyer 1977 see Kraft & Ackerly 2013).

Abiotic and biotic factors create filters, which species that tend to colonize or establish at a site have to pass through according to their traits or which species that still survive/persist at this site has to interact with (Harper 1977; see Hobbs & Norton 2004). Note that biotic interactions can occur in three different ways. Biotic interactions can be negative, e.g. when plant species compete for same resources or if there are insect attack (Grace & Tilman 1990) or predation (Olff *et al.* 1999). Biotic interaction can also be positive or neutral. Positive influences are facilitation as in nurse plant (e.g. spending shadow (Franco & Nobel 1989)) and of nitrogen-fixing legume species (N sparing, N transfer (decomposition, exudation, direct transfer via mycorrhizae)) (Conell & Slatyer 1977, Callaway 1995, Temperton *et al.* 2007), or mutualism when both partners benefit from the interaction in some way (Withgott 2000) or during pollination.

Priority effects

Therefore, in assembly, the species or species traits that establish first at a previously disturbed site can play a key role in the further development of the community and can strongly influence plant community composition at multiple spatial scales (priority effects) (Young *et al.* 2001, Fukami *et al.* 2005, von Gillhaussen 2015, Weidlich et al. 2017). The establishment of species, however, is controlled by the respective conditions at the time when this temporal window is opened. Priority effects cause historical contingency, which is the effect of the order and timing of past events on community assembly (like disturbance such as floods, fires, storms, and earthquakes or the arrival of species) in the structure and functioning of communities, which can than result in alternative stable states, alternative transient states or compositional cycles (Fukami 2015). Historical contingency has historically been ignored often in ecology but increasingly it is seen as being a potentially key driver of community diversity and ecosystem functioning (Brudvig 2011, Grman *et al.* 2013).

The consideration of the possible role of priority effects is therefore important in the restoration of degraded sites (Bullock *et al.* 2001 and 2007) and invasion ecology (Cleland *et al.* 2015). Priority effects can be defined as follows: Priority effect is the impact that a particular species can have on the further development of the community due to prior arriving at a site or if the establishment or colonization of the early-arriving species in an ecosystem significantly affects/influence the establishment, growth, or reproduction of later-arriving species (Facelli & Facelli 1993, Young *et al.* 2001, Fukami *et al.* 2005, Grman & Suding 2010). Priority effects may be the main mechanism underlying the impact of colonization history in plant community structure (Young *et al*. 2001, Fukami *et al*. 2005). Priority effects

can lead to lasting differences in the legacy of species or functional group dominance (Fukami *et al.* 2005, Körner *et al.* 2008), successional trajectories (Chang & Turner 2019), and hence can potentially drive ecosystem properties and functioning (Bullock *et al.* 2007, Körner *et al.* 2008, Tan *et al.* 2012, Fukami 2015, Sarneel *et al.* 2016, Weidlich *et al.* 2017).

Some authors consider priority effects caused by different sowing events in management or ecological restoration to only occur when two sequential sowing/introduction events are undertaken (*priority effect 2* e.g. Fukami 2015, Weidlich *et al.* 2017). In a sense however, biodiversity ecosystem functioning (BEF) experiments where different communities are sown at the same time (but it is also depended on species specific germination time, not all sown species germinated at the same time), and in those where further immigration of species is allowed (where weeding is stopped), this can also be seen as a form of priority effect (*priority effect 1*, Bullock *et al.* 2001, 2007).

Therefore, priority effects can be experimentally initiated and identified by sowing *different* initial seed mixtures *at the same time (herewith defined as priority effect type 1, Manuscript 2, to test how starting biotic conditions affect overall trajectories of vegetation*, see also section 1.1.3*)*, or by sowing the *same* seed mixtures or functional species group at *different time of arrival (priority effect type 2, Manuscript 3,* see also section 1.1.4*)*.

Priority effects can occur on timescales from days to years. They can be linked to differences in the arrival of a species at a site but also to their success in establishing themselves in the community (attributes of species after arrival) and their persistence. Positive (facilitative priority effect) and negative (inhibitory priority effect) interactions between organisms can in turn influence how strong priority effects are. Delory *et al.* (2019) found that moving from negative to positive priority effects increased grassland overyielding, indicating the need to now also assess whether priority effects are negative or positive. The strength of priority effects also differs depending on soil nutrient content as well as on plant soil feedback (van de Voorde *et al.* 2011, Kardol *et al.* 2013). Therefore, priority effects can explain successful or non-successful invasion or colonization of species in cleared or degraded areas (Walker, Walker & Hobbs 2007). Fukami (2015) highlighted that "the mechanisms of priority effects fall into two categories, niche preemption (asymmetric competition, early arrivers draw down a common resource) and niche modification (plant-soil feedback, change the environment in a way that alters the success of later arrivers), and the conditions for historical contingency by priority effects can be organized into two groups those regarding regional species pool properties and those regarding local population dynamics". Historical contingency includes order of arrival of specific organism as well as effects of disturbances (Fukami 2015).

Historical contingency is on the one hand a random factor in assembly, but if humans intervene and sow different mixtures or different invasion sequences (priority effect 1 and 2) it can be used as a steering factor and has the potential to identify the extent of random versus deterministic drivers. Historical contingency is often resource related (Chase 2003, 2010, Kardol *et al.* 2013, Conradi *et al.* 2017) and size dependent (Drake 1991). But the range and the effect size along temporal, spatial and resource gradients and their persistence are rarely addressed.

Foster & Dickson (2004) hypothesize that systems with higher resource availability (availabilities of establishment microsites and water were manipulated) exhibit more available niches but these niches are usually packed with species. Species populations are thus subject to enhanced neighborhood competition. In contrast, in systems with limited availability of resources such as soil nutrients, establishing species generally find more open niche space due to the reduced neighborhood competition, but mainly face the limitation posed by abiotic conditions, which is similar to the concept of the abiotic filter in filter theory (Hobbs & Norton 2004).

Trait based approaches

In recent years, the research focus in community ecology shifted from species-based to a more trait-based (*Manuscript 4*) view (McGill *et al.* 2006). Several studies have investigated community assembly processes with the view that species are filtered by the environment according to their traits and that these functional plant traits form the basis to understanding assembly mechanisms, especially the effect of environmental filters structuring plant communities (Weiher & Keddy 1995, Diaz *et al.* 1998, Jung *et al.* 2010, Lebrija-Trejos *et al.* 2010, Kraft *et al.* 2015). Plant functional traits are defined as morphological, physiological and phenological characteristics that directly or indirectly affect individual performance and fitness of a species (Cornelissen *et al.* 2003, McGill et al. 2006, Violle et al. 2007, Klimešová *et al.* 2019). The definition of traits in general is not always straightforward and remains ambiguous. In the ecological literature functional traits have been viewed and applied in numerous ways (Nock *et al.* 2001, Violle *et al.* 2007, Diaz *et al.* 2013).

In consequence, trait-based community assembly can be understood to be driven by two distinct selective processes of species sorting: habitat filtering and niche differentiation (Weiher & Keddy 1995, Grime 2006, Jung *et al.* 2010). Trait-based analyses of plant communities often focused on mean trait values across species and interspecific trait differences (Weiher & Keddy 1995, Stubbs & Wilson 2004, Ackerly & Cornwell 2007). But

the neglect of intraspecific trait variation (*Manuscript 4*) may lack much of the spatial and temporal variation in community trait distributions and therefore much of the interaction of community assembly and ecosystem functioning (Siefert 2014, Chalmandrier *et al.* 2017).

1.1.2 Community assembly and priority effects in a grassland restoration context

Semi-natural grasslands are hot-spots of European biodiversity with up to 80 species per m^2 (Peet *et al.* 1983, Wilson *et al.* 2012). Semi-natural grasslands are also the vegetation type with the highest diversity of species per m^2 world-wide e.g. oligo- to meso-trophic, managed, semi-natural, temperate grasslands 89 species on $1m^2$ (Argentina), tropical rainforest 942 species on 1 ha (Ecuador) (Willems *et al.* 1993, Wilson *et al.* 2012). The conservation and restoration of species-rich grasslands is a high priority currently in European policy, in terms of maintenance of diversity and functioning (Silva *et al.* 2008). Because of climate changes areas of central and northern Europe are expected to become more arid in parts and as such, dry or calcareous grasslands are predicted to possibly increase under climate change comparison to more mesic grasslands (Hufnagel & Garamvölgyi 2013). Calcareous grasslands have proven to be resistant to short-term drought, but Basto *et al.* (2018) could show that long-term drought effects on calcareous grasslands have larger impact than previously thought. In addition, species-rich grasslands are currently threatened by both intensification and land abandonment which has led to a drastic decrease in area over the last few decades (Kirmer *et al.* 2012).

With regard to restoration ecology, which involves the creation of new communities of conservation value on bare or degraded sites (Bullock *et al.* 2007), the reestablishment of ecosystem services and species diversity on degraded land is of great interest (Hobbs & Harris 2001).

Community ecology is frequently used as complementary to and useful for guiding ecological restoration (Wainwright *et al.* 2018), because it describes the processes that underlie the assembly (Diamond 1975, Fukami & Nakajima 2011), maintenance of diversity and functioning of ecological communities (Bullock *et al.* 2001, Balvanera *et al.* 2006, Isbell *et al.* 2011) which are often the focus and primary objectives of ecological restoration (Zirbel *et al.* 2017, Wainwright *et al.* 2018). Young *et al.* (2001) wrote that: “In particular, two conceptual models in community ecology have relevance to ecological restoration: 1) community succession, which dates back more than a century (Cowles 1899) and 2) the more recently developed ideas of community assembly and priority effects (Palmer *et al.* 1997, Lockwood 1997)”.

The key to successful restoring degraded ecosystems is to understand how ecosystems are

assembled (deterministic, stochastic, ASS model), which mechanism are behind the pattern you observe and in particular how the species that make up a particular biological community arrive in an area, survive, and interact with other species in dependency of the abiotic conditions (filter theory) (Temperton *et al.* 2004).

Both the diversity as well as the history of species (priority effects/arrival time) can have strong effects on ecosystem functioning and long-term community composition (structure). Fukami *et al.* (2015) reviewed that priority effects can have a stronger influence on community composition than abiotic conditions.

Priority effects, the initial plant species composition (diversity) and the order of arrival can be manipulated in ecological restoration and for example may be useful during restoration to steer plant communities toward desired outcomes (Schantz *et al.* 2015, Vaughn & Young 2015, Temperton *et al.* 2016, Weidlich *et al.* 2017).

Therefore, a better understanding of the mechanisms which control community assembly, especially priority effects (see the next sections 1.1.3. and 1.1.4), is central to understanding ecosystem functioning, the maintenance of biodiversity and to informing restoration of species-rich grassland types.

1.1.3 Grassland biodiversity experiments without weeding, natural assembly (priority effect 1)

In communities with unknown assembly history the long-term effects due to priority effects and the role of dispersal limitation are hard to detect (Roscher *et al.* 2014). For that case, biodiversity experiments generate a good possibility to study such biotic processes of community assembly, because abiotic (similar environmental conditions between plots) and biotic (assembly history/different plant diversity between plots) effects are controlled and well known (Allan *et al.* 2013, Roscher *et al.* 2014).

Tillman *et al.* (2014) reviewed that from Biodiversity experiments it is known that: “species diversity is a major determinant of ecosystem productivity, stability, invasibility, and nutrient dynamics. These impacts of higher diversity have multiple causes, including interspecific complementarity, greater use of limiting resources, decreased herbivory and disease, and nutrient-cycling feedbacks that increase nutrient stores and supply rates over the long-term“.

We now know a great deal about the often positive effects of plant diversity on ecosystem functions such as productivity from biodiversity-ecosystem functioning experiments (Balvanera *et al.* 2006, Cardinale *et al.* 2006). Such experiments involve artificially maintaining the desired species or functional group richness and randomly selecting species from a defined species pool, since here the main questions relate to effects of diversity *per se*.

In more natural communities, factors other than diversity, such as land management, fertility of soils, climatic conditions, history or invasive species, are often considered more important key drivers of ecosystem properties. In more open, natural communities, undergoing natural assembly and succession, however, only few studies that have addressed how important diversity effects are in relation to other ecosystem drivers (Flombaum & Sala 2008, Tylianakis *et al.* 2008).

The following grassland experiments with natural assembly after manipulating starting biodiversity are examples to show the influence of different starting diversity (priority effect 1) on community assembly over time. In a grassland restoration context, Bullock *et al.* (2001, 2007) sowed either low or high diversity mixtures using plant mixtures typical for UK grassland restoration on a whole series of ex-arable sites and over a long period of time. Both studies found long-lasting effects of initial sowing of seeds (which I call priority effects 1 from now on) and strongest effects when more diverse seed mixtures were sown.

Fukami *et al.* (2005) manipulated in a 9-year grassland experiment the initial plant composition by sowing different diversity seed mixes (zero, four and 15 species) on abandoned arable land and subsequently allowed natural colonization. During community assembly they analyzed if communities converge in their species and trait-group composition. They found out that the answer depends on the level of community organization and that the initial compositional variation was still affecting community composition (divergent in species identities) but species traits converged over time.

Bezemer & van der Putten (2007) sowing either zero, four or fifteen species of plants onto ex-arable land then followed the dynamics of the system in terms of species turnover but also productivity, temporal stability and diversity in terms of species richness, and Shannon diversity index.

Flombaum & Sala (2008) removed species to create a plant species diversity gradient in the Patagonian steppe and found that aboveground net primary production increased with the number of plant species.

Roscher *et al.* (2009 and 2014) also focusing on natural colonization of new species in never weeded subplots compared to weeded subplots after sowing different diversity levels (included in the Jena experiment, species richness from 1 to 60 and plant functional group richness from 1 to 4) and monitored species richness and composition (abundances of sown species, community invasibility) and different components of functional diversity (productivity).

An important emerging insight from studying effects of plant diversity on a whole range of response variables is that the concept of multifunctionality (*Manuscript 1*) (Hector & Bagchi 2007, Zavaleta *et al.* 2010, Lefcheck *et al.* 2015) needs taking into account when applying research outcomes to natural communities. When considering a range of environmental change scenarios, different species promote ecosystem functioning at different times and for different functions (Isbell *et al.* 2011).

Many large biodiversity- experiments on grasslands have a high nutrient availability in the soil, and we still no very little about the relationship between diversity and ecosystem processes in more dry or arid or nutrient-poor systems, which I want to analyze with *Manuscript 2* (but see Pugnaire *et al.* 1996, Baasch *et al.* 2012).

Plückers *et al.* (2013b) (*Manuscript 2*) tested in a grassland experiment whether sowing two different diverse seed mixtures at time zero onto a sandy substrate (nutrient-poor system), which formed an equivalent to a primary succession would create any priority effect over time. We found out that priority effects did also occur in dry acidic grasslands but how persistent they were over time depended on the response variable considered. In contrast to a more nutrient-rich treatment the breadth of responses affected may not be as wide but however after 4 years the sowing event were still visible for aboveground productivity and also for functional composition of the community but species richness varied strongly each year.

1.1.4 Grassland experiments with natural assembly after manipulating plant species order of arrival (priority effect 2)

Sowing seed mixtures on empty ground may help overcome dispersal barriers in grassland system especially in dry acidic grasslands in a restoration context but a central question is, what effect it has if viable seeds of several species are sown at one date (priority effect 1 (see also section 1.1.3)), compared with a variable arrival and germination (priority effect 2 (this section)), as is more the case in nature. This variability in arriving and germination can change the assembly path in a different way and influence species establishment, because differences in arrival time and development can create a very particular competitive situation compared with one sown date, where all species have the potential to germinate at on common date and stand directly in competition (Körner *at al.* 2008). History of species arrival (order and timing) can influence plant community assembly (Fukami 2015). Grassland experiments with natural assembly after manipulating plant species (plant functional groups) order of arrival (priority effect 2) can be another way to study community assembly with known community history as you can see in the following presented studies.

In the United States experiments on priority effects in plant communities found that the role of order of arrival of invasive exotic annual grasses, often originating from Europe, played a key role in affecting performance of native species (Martin & Wilsey 2012, Goldstein & Suding 2012, Vaughn & Young 2015). Vaughn & Young (2015) for example could show that a two-week planting advantage significantly increased the establishment success of native perennial grasses which is important for native grassland restoration in California. In species-rich grasslands in Europe, however, the main threat is land use intensification as well as abandonment and so the maintenance of diversity and productivity are in focus (Bullock *et al.* 2007).

Ejrnaes *et al.* (2006) and Kardol *et al.* (2013) manipulated, in grassland microcosm experiments, soil fertility and arrival order of species. They found that the arrival order of species affected community assembly but in depends on the trait one measured. The size of the effects depended on soil fertility and was depended on the measured trait.

Körner *et al.* (2008) tested in a pot experiment (glasshouse and containers outside) the effect of species arrival by stepwise sowing calcareous grassland species of different plant functional types and found strong priority effects on above- and belowground productivity when legumes were sown before the other functional types. A 3-week arrival difference had a dramatic effect on composition and aboveground biomass that persisted over four harvested and two seasons. This effect may seem surprising considering that all other factors were held the same – only the order of arrival was manipulated and it very significantly affected productivity and the effect increased over time.

Von Gillhaussen *et al*. (2014) (*Manuscript 3*) tested the effect of sowing plant functional types in a different order in combination with a sowing density treatment in a greenhouse experiment. We found that the order of arrival of different plant functional types had a much stronger influence on aboveground productivity than sowing density or interval between the sowing events. The sowing of legumes before the other two functional groups affected productivity the most.

Roscher *et al.* (2014) also addressed assembly questions (assembly history) within a grassland biodiversity experiment, the Jena Experiment by stopping weeding and adding seeds. After 5 years historical contingency could not eradicated by stopping to weed or forced colonization with regard to realized species composition.

Weidlich *et al.* tested in their grassland field experiment the effect of order of arrival of three plant functional groups and of sowing low and high diversity seed mixtures on species composition and aboveground biomass (2017) and the root development (2018) in two

different soil types. The order of arrival affected aboveground productivity, the number of species and community composition. Sowing legumes first created higher aboveground productivity but was not continuous over time. They also found in Weidlich *et al.* (2018) that sowing legumes first may have created a priority effect with a lower standing root length density in the first and third year, even though the functional group dominance was different in each of the studied years.

1.1.5 Grassland experiments in community assembly with trait approaches (intraspecific variability)

On the one hand for explaining community dynamics it is important which species or functional group arrive first (priority effects), but on the other hand it is maybe more crucial which functional traits these species have (Fukami *et al.* 2005, McGill *et al.* 2006). Trait-based approaches are useful for addressing and understanding the mechanisms controlling the coexistence of plant species and how plant communities are structured across environmental gradients (Lavorel & Garnier 2002, McGill *et al.* 2006, Ackerly & Cornwell 2007, Violle *et al.* 2007, Webb *et al.* 2010, Violle *et al.* 2012). Because plant functional traits are related to species niches (Thuiller *et al.* 2004) and therefore, the variation of these traits among and within species within communities (functional diversity) can reflect the effect of environmental filtering or competitive interactions (Chalmandrier *et al.* 2017). Interactions with the biotic and abiotic environment are ultimately based at the level of the individuals within and among species. Priority effects therefore also interact with phenotypic plasticity of the individuals.

However, recent studies have shown that the intraspecific trait variation is important for the maintenance of species coexistence and also influences functional community composition (Albert *et al.* 2010 a and b, Messier *et al.* 2010, Albert *et al.* 2011 and 2012, Violle *et al.* 2012, Siefert *et al.* 2015). Most new approaches measure intraspecific trait variation in relation to surrounding biodiversity, along environmental gradients, such as flooding or drought, under different climatic scenarios, during community assembly or between different regions/local scale (between different populations of the same species) (Jung *et al*. 2010, Beierkuhnlein *et al.* 2011, Lemke *et al.* 2012, Wellstein *et al.* 2013, Jung *et al,* 2014, Lipowski *et al.* 2015, Siebenkäs *et al.* 2015, 2016, Siefert *et al.* 2015, Roscher *et al.* 2013, 2018 a and b).

Intraspecific trait variation can be large in response to abiotic and biotic effects (Albert *et al.* 2010 a, Violle *et al.* 2007, Jung *et al.,* 2010, Mitchell & Bakker 2014). Several studies show that the amount of intraspecific variation differ between species and traits (Siebenkäs *et al.*

2015, Siefert *et al.* 2015), but very little is known about how intraspecific trait variation is general structured both spatially and temporally (Albert *et al.* 2010 a, Buchmann *et al.* 2017), because intraspecific trait variation is influenced by different mechanisms (Albert *et al.* 2010 a, Messier *et al.* 2010, Buchmann *et al.* 2017).

This requires a focus both on comparisons of mean values between treatments as well as on how variable those mean values are across space and time especially during assembly (Siefert 2014). The following studies are examples for the important of measuring or taking intraspecific trait variation by analyzing community assembly into account.

In the meta-analysis of Siefert *et al.* (2015) they conducted the relative extent of intraspecific trait variation within and among plant communities worldwide. They found that with increasing species richness and spatial extent, the relative amount of intraspecific trait variation decreased, but this did not vary with plant growth form or climate. They highlighted that their results showed "global patterns in the relative importance of intraspecific trait variation in plant communities, providing practical guidelines for when researchers should include intraspecific trait variation in trait-based community and ecosystem studies".

In the study of Buchmann *et al.* (2017) they found that traits of two grassland species responded to within site conditions, whereas one did not, and recommended further research to test this for more species.

In the study of Roscher *et al.* (2018a) they analyzed in the Jena Experiment means, extent of variation and plasticity to increased plant diversity for nine functional traits to identify whether species classified into functional groups based on interspecific trait differences show similar patterns of intraspecific trait variation regarding in response to varying plant diversity and composition in local communities. Their results suggest that "the concept of functional groups is viable, but context-specific trait measurements are required to improve our understanding about the functional significance of intraspecific trait variation and interspecific trait differences in local plant communities".

Roscher *et al.* (2018 b) studied functional traits and plant biomass of 59 species in experimental grassland mixtures in the Jena Experiment of varying species richness. They analyzed mean species performances and also their trait plasticity and trait differences to other species. They highlighted that their results "suggest that incorporating plasticity in trait expression as well as trait differences to co-occurring species is critical for extending trait-based analyses to understand the assembly of plant communities and the contribution of individual species in structuring plant communities".

2 Objective and outline of manuscripts

The objective of this thesis is to describe community assembly especially in dry acidic grasslands using different factors to elucidate the assembly mechanisms. Analyzed factors (environmental gradients) considered in this thesis are: historical effects/different starting conditions in neighborhood or diversity (in particular priority effects 1 and 2), substrate (abiotic filter effect) and time (year effect). Community and specific plant species traits were analyzed (species and trait-based approaches) with appropriate ecological methods in order to trace changes in community structure under these different factors.

With each study the changes in community assembly were analyzed from a different perspective or different ecological approach (Figure 2, community level, plant functional group level, specific plant traits level). These analyses are used to gain a better understanding of the different mechanisms on different levels of community assembly itself over time.

The design of the studies can be allocated to basic ecological research but the results contribute to the field of applied ecology and improve the understanding of community assembly and its relevance to restoration of degraded landscapes.

The two superordinated questions for the studies conducted within the framing of this thesis are:

1. How do the different factors influence community structure?
2. In which trait changes are those effects detectable?

The thesis is mainly based on a field experiment entitled the Habitat Garden Experiment (see Plückers *et al.* 2013a and b), in which assembly in semi-natural grassland communities is analyzed in the context of restoration. The experiment was established in autumn 2007 on the campus of the Forschungszentrum Jülich, located in Jülich, West Germany (6_ 220000E, 50_ 560000N), and consisted of 12 plots, 6 dry acidic grassland plots with two different diversity mixtures (2 grass and 25 forbs versus 7 grass and 32 forbs) and 6 mesic plots where the same diversity mixtures are sown. The main focus is on the dry acidic grassland plots where the effect of sowing initial seed mixtures at the same starting time is investigated

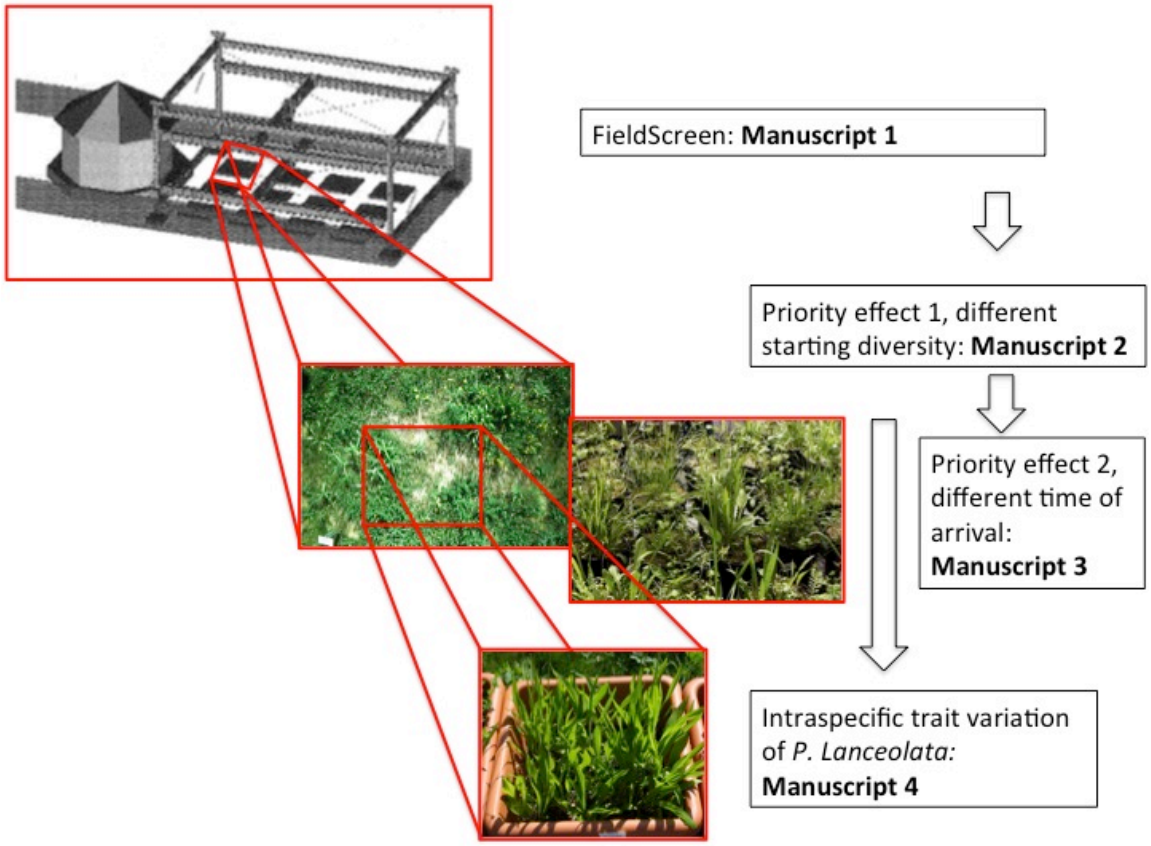

Figure 2. Schematic view of the connection between the manuscripts contributing to this thesis. With each phase different ecological approaches are addressed. Starting point is the Habitat Garden Experiment, Manuscript 1: FieldScreen community traits measured non-invasive over time, Manuscript 2 and 3: community traits measured invasive in question to priority effects over time, Manuscript 4: specific plant traits measured in question to priority effect 1, substrate and neighborhood over time.

The individual manuscripts of this thesis are the following:

Manuscript 1

This study tested a new approach to address general research questions on multifunctionality and to detect changes in ecosystems over space and time non-invasively.

This new approach is a Mobile Field Positioning System called "FieldScreen", which accurately positions a sensor and enables automated and repeated non-invasive measurements of plants and soil surfaces.

This automatic mobile positioning field system was set up over the main Habitat Garden Experiment. The aim was to link community assembly and physiological research with an automated non-invasive positioning system for measuring multiple traits of vegetation in the field at different time scale. Ecosystem and community assembly are subjected to dynamic changes at many different spatial and time scales.

The set-up focuses on obvious visible differences between the dry acidic grassland plots with different initial starting conditions (priority effect 1) over the course of several years with the

help of this kind of measurements and present the strengths and possibilities of this new approach.

The following question was asked:

What kind of ecological or physiological traits can be followed using the FieldScreen at community and species-level and are differences in community assembly under different starting conditions (priority effects 1) detectable over time?

This study showed in the first 3 years of observation that with the FieldScreen it is possible to non-invasively detect changes of species turnover and selected plant traits over time. With the photos taken with a camera mounted on the FieldScreen trolley it can be clearly distinguished that sowing initially different diversity levels (priority effect 1) has an abiding influence on the further development of the plant communities, the spatial spread of species and the overall vegetation cover.

These time series have the potential to address research questions on the dynamic nature of ecosystem functioning. This could include measuring several traits of plants at the same time and hence helping to address the need to measure multifunctionality in natural systems if we are to better understand how diversity and ecosystem functioning are linked in natural systems subjected to many disturbances and drivers.

This combination of traditional and high-tech methods will allow very detailed analysis at much high spatial and temporal resolution than is possible using traditional ecological methods for assessing plant community change over time (e.g. assessing groundcover of plants by eye).

Manuscript 2

This study investigated the effect of sowing different initial seed mixtures at the same time (priority effect 1) on productivity, richness and composition in community assembly of dry acidic grassland communities in the Habitat Garden Experiment over time. The analyses are done with a view to restoration applications and thus formed an equivalent to a primary succession. The second manuscript examined the analyses of the detailed traditional ecological measurements of functional ecosystem traits (community level/ species level) in the Habitat Garden Experiment in the dry acidic grassland plots over time. In order to test this priority effect 1 over time, community trait changes between the plots are followed over 4 years.

Response variables measured are: species number, species cover and total aboveground peak biomass as a surrogate of productivity, in total and split into functional groups. Also

differences between responses of target (desired sown) and non-target species (invaders) are assessed.

This field study aims to test the strength of priority effects 1 in dry acidic grasslands over time.

Particularly, the following questions were asked:

Does sowing two different seed mixtures produce priority effects in dry grassland, and how sustainable are they over time?

If there are priority effects which traits, processes or characteristics of the ecosystem do they relate to most?

Priority effects of sowing even four years after the start of the field experiment were found, but how sustained they were depended on the response variable measured. Aboveground productivity, cover and functional group composition were still significantly affected by the sowing treatments four years later, whereas species richness was not. This study found relatively low establishment success of target species, but the results are in line with results from low-nutrient grassland restoration, suggesting that microsite limitation and related filtering effects of severe abiotic environments rather than biotic interactions may be the strongest driving factors in assembly of dry acidic grassland. This study is unusual in that most priority effects studies have focused on more nutrient rich soils, and there the priority effects found were generally wider in breadth of response variables affected than in our dry acidic grassland study.

Manuscript 3

This study investigated the effect of order of arrival (priority effect 2) of different plant functional types on the productivity as well as species and functional composition of species-rich grassland communities grown in pots under greenhouse conditions. The experiment tested the effects of order of arrival, density and sowing interval on community productivity and composition.

The response variables measured were: aboveground biomass, cover and number of individuals per plant species.

The following questions were asked:

How do the sowing interval between the plant functional type sown first and the subsequently sown plant functional types influenced the aboveground productivity of the system?

How does sowing different seed densities result in individual numbers and overall aboveground productivity?

Aboveground biomass (community productivity) increased when legumes were sown first but the priority effect of order of arrival was larger in effect size than any sowing interval or sowing density effect. Size asymmetric competition was considered to be the main mechanism behind the observed priority effect, which promotes the plant functional type sown first in respect to later sown plant functional types. In the legume-first treatment smaller rooting systems of legumes allowing better root foraging of the later arriving plant functional types.

Manuscript 4

This study investigated the effect of different factors (priority effect 1, substrate, neighborhood) on the variation in aboveground traits of *Plantago lanceolata* (trait-based approach) both under mesocosm and field conditions (Habitat Garden) over time during community assembly.

Plant traits under consideration were: leaf dry weight, leaf area, specific leaf area, leaf nitrogen and leaf chlorophyll *a*.

These trait data were collected under more controlled conditions as the plants were grown outdoors in pots (mesocosm), so that they experience similar environmental conditions as the field plants. They will provide an idea of the environmental amplitude of the species in question, and can be related to possible turnover of species in the field when environmental conditions there change (i.e. accumulation of N in soil).

Functional traits of *P. lanceolata* growing on different substrates and with different neighbors under both mesocosm and field conditions were measured over time. Also the trait variability (the coefficient of variation, CV) within experiments and between experiments (mesocosm vs. field) was compared.

Trait variation was defined as an umbrella term for both the differences between trait means of populations across a range of treatments as well as the variability of trait values. Variability was defined, in contrast as a measure of trait dispersion, the relative amount of trait variation around the population mean e.g. the variability of trait values measured as the coefficient of variation.

The following question was asked:

Is species' trait variation (mean trait differences and trait variability) similar under varying site conditions (substrate, neighborhood, time and experimental set –up) in *P. lanceolata*?

In mesocosms, traits responded as expected much more to soil fertility (substrate type) than to neighboring species, but trait values in more fertile and less fertile substrates converged over

time. In field settings soil fertility had less influence on trait differences, however some leaf traits responded to legume cover. We found high trait variability in the low fertility substrate and much less variability in the more fertile soils in the mesocosms. The tested conditions had more influence on differences in mean trait values and trait variability in the mesocosms than under field settings. Traits were more stable under different environmental conditions in field settings. This suggests, as hypothesized, that less favourable, more stressful sites may favour higher phenotypic plasticity. Multiple concurrent factors as found under field conditions can lead to a more conservative phenotypic range.

3 Overall research questions and summarizing conclusion of the thesis

The superordinated questions on which this thesis is based on is what role does sowing specific seed mixtures either simultaneously (priority effect 1) or at different time points (priority effect 2) play for both biodiversity and ecosystem functioning outcomes in semi-natural grasslands especially in dry acidic grassland? How nutrient availability modulate priority effects? How can the outcomes be used for ecological restoration? In detail whether and how priority effects in relation to soil nutrient availability (abiotic filter effects) influence community structure and stability in function of time and in which functional trait changes (variation in community function traits (productivity, composition, cover, richness) and variation in plant species trait) are those effects detectable and useful for ecological restoration?

The role of historical contingency in forming alternative vegetation states is attracting increasing attention (Brydvig 2011, Grman *et al.* 2013), with priority effects producing long-term and significant effects on ecosystem functioning and diversity (Sarneel *et al.* 2016, Weidlich *et al.* 2018). The role of nutrient availability in modulating the strength of priority effects is an important consideration, since significant effects of order of arrival on communities may depend strongly on how many nutrients are available in the soil (Chase 2003, Kardol et al. 2013). The range and the effect size of these key drivers (e.g. historical contingency, nutrient availability) of assembly along temporal, spatial and resource related gradients are rarely addressed. Natural systems are subjected to dynamic changes at many different spatial and time scales, which can influence vegetation states. The outcomes of field experiments in community ecology differ with variation between years and sites (Bakker *et al.* 2003, Vaughn & Young 2010). This highlights the importance of measuring or better exploring spatial and temporal trajectories at various scales to detect differences in assembly. Especially in times of global change it gives the chances to let the results of ecological experiments be general and not unique to a particular site or time (Coreau *et al.* 2009, Young *et al.* 2015, Temperton *et al.* 2016). Figure 3 and Figure 4 show the different processes and drivers which might influence community assembly and highlights the embedment and the connection of the four manuscripts of this thesis on it.

The investigation of temporal dynamics in community assembly of dry acidic semi-natural grasslands with a new automated field measurement system the FieldScreen *(Manuscript 1*/Plückers *et al.* 2013a) demonstrated that there are dynamic changes within the vegetation

cycle on short time periods (alternative transient states only stable for a limited time) which we can miss with classical ecological assessments. With this technical approach the identification of single species, especially dominant species and plants traits, prevalent the phenology (flowering time) over time was possible, but it was not possible to identify every single species or the degree of coverage of every single species in this highly diverse system like conventional ecological assessments can. Nevertheless, for research on open ecological systems, FieldScreen is a useful tool to follow species spread and invasions continuously with much higher observation frequencies than conventional techniques. Continuous ecological monitoring systems, such as the FieldScreen have the potential to reveal novel properties during assembly (measuring multifunctionality).

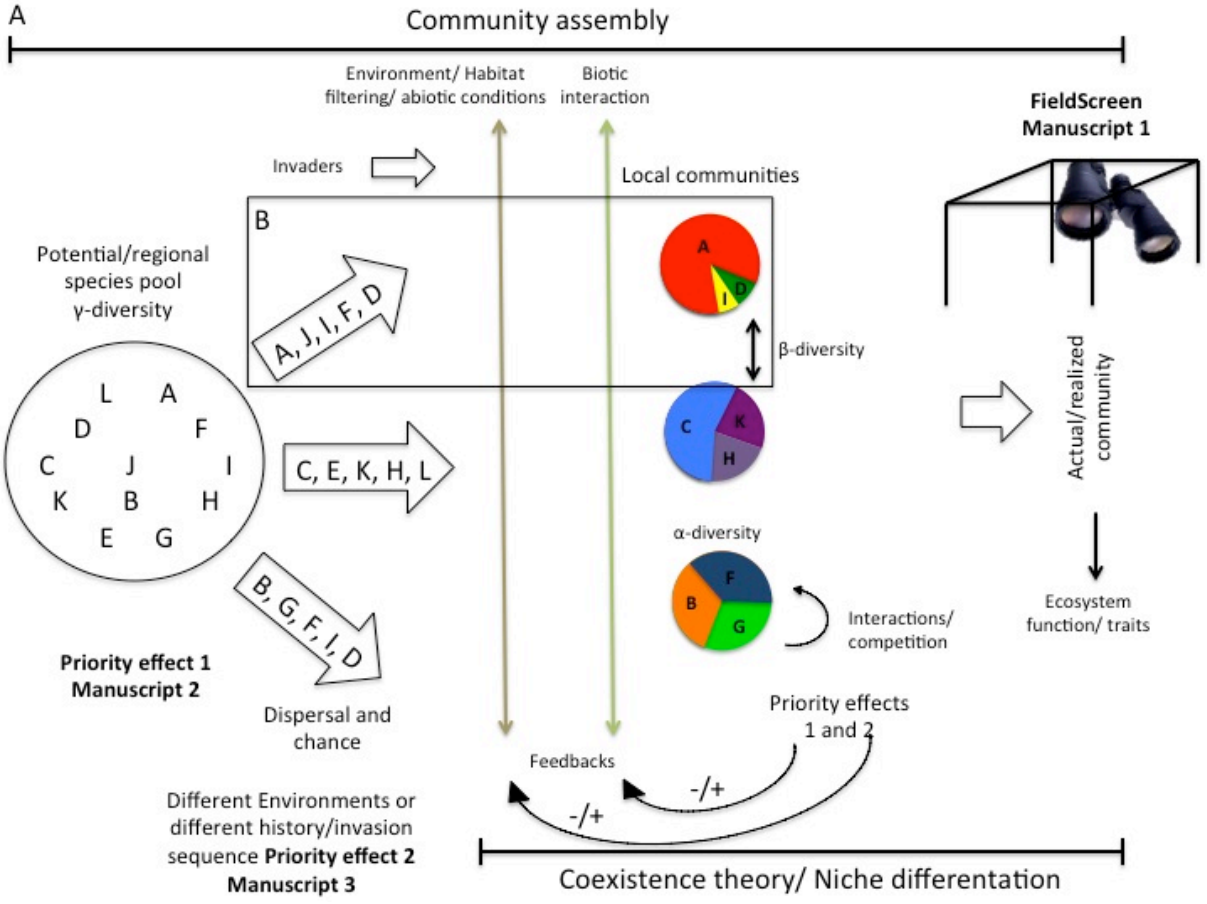

Figure 3. A) Schematic view of the different processes which might influence community assembly and highlights the connection between the four manuscripts (adapted from HilleRisLambers *et al.* 2012). "Community assembly is influenced by processes operating at a wide range of spatial temporal scales. Species belong to a regional species pool (y-diversity) that is constrained by historical processes (including evolution). A subset of the regional species pool (influenced by chance and dispersal limitation) is available for colonization of a particular site." At this particular local site, habitat filtering and biotic interactions (competition, facilitation, niche preemption, niche modification/differentiation) define the actual local assemblage of plant species according to their traits (alpha diversity, beta diversity is the result of different environmental filters or invasion sequences (historical contingency/Priority effects)). **B)** Highlights if you also include intraspecific trait variations see details in Figure 4.

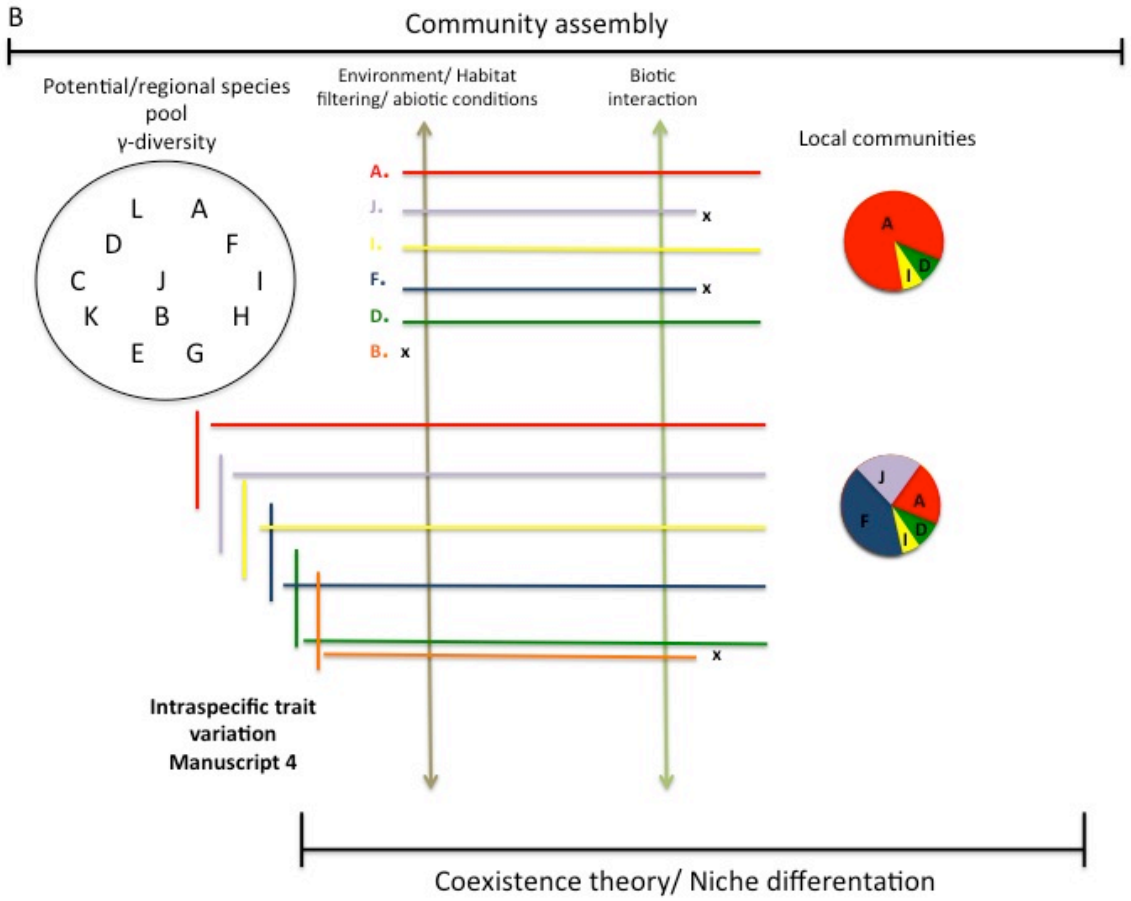

Figure 4. B is part of Figure 3 A. Schematic view if you not only include species perse but also their intraspecific trait variation in reaction to the environmental conditions. Plant traits can be addressed in two different ways: variation in traits among (interspecific/between) and within species (intraspecific). The intraspecific trait variation can be large in response to abiotic and biotic effects and allow certain species to pass through the mesh and establish. Because plant functional traits are related to species niches (Thuiller *et al.* 2004) and therefore the variation of these traits among and within species within communities (functional diversity) can reflect the effect of environmental filtering or competitive interactions (Chalmandrier *et al.* 2017).

During natural succession/assembly, systems at the beginning are often limited by dispersal and in more nutrient-poor sites also by microsite limitation. To reduce or to avoid such influencing factors like microsite limitation and dispersal during experimental assembly but also let other influencing factors like abiotic and biotic filter effects happen, sown grasslands with known historical factors are good study systems to test priority effects (historical contingency).

The main grassland field experiment (*Manuscript 2*/ Plückers *et al.* 2013b) displays the temporal dynamics of community assembly in dependency of priority effect 1 and limited soil nutrient availability in the closer context of restoration applications. The priority effect of sowing different initial seed mixtures at time zero simulate differing dispersal filters and history of species, which are seen as one intersection between potential species pool (gamma diversity) and realized species pool (alpha diversity) (Figure 3). Our results showed that there

is a priority effect and do play a role in community assembly of dry acidic grasslands, but the stability over time depended on the variables measured, with stable priority effects being found in relation to community aboveground productivity and plant functional group composition but not for community composition or species richness. These results supported the findings of Sutherland (1974), that the vegetation in some ways reached a stable state in terms of the plant functional group relative composition but not in terms of species richness of species composition.

The low nutrient availability negatively influenced the establishment of target species (40% in the fourth year), but the results are in line with results from low-nutrient grassland restoration. The breadth of responses of priority effects is depended on nutrient availability because it was not as wide in their effects as those found in mesic grasslands. Both points suggested the important role of abiotic factors (microsite limitation and related filtering effects) on community assembly. Other experiments on ex-arable land with higher resource availability could shown that initial sowing (priority effect 1) significantly affected aboveground biomass (productivity) (Bullock *et al.* 2001, Bullock *et al.* 2007) and other ecosystem functions such as stability, arthropod diversity or invasion resistance (Dedov *et al.* 2006, Bezemer & van der Putten 2007, Roscher *et al.* 2009). Kardol *et al.* (2013) also pointed out in their study that the timing of species arrival had a large impact on community assembly, but the size of the effect depended also on soil fertility. They also explained that asymmetric competition is the most plausible explanation for this effect.

Furthermore it could be shown that not the species richness of the initial seed mixture was the determining factor but functional composition especially the plant functional group of legumes have an abiding positive effect on community traits (aboveground productivity, cover and a higher establishment of target species) in these grasslands, which could be also demonstrated using the FieldScreen (*Manuscript 1*). This indicate that reduction of microsite limitation via planting out nurse plants to facilitate establishment in such harsh conditions or including an intermediately severe disturbance regime (as in Jentsch *et al.* 2009) may be as important to improving dry grassland restoration success as sowing therefore.

This study leads to emerging recommendations and gives further direction in:

1) Dynamic changes of the system should be followed over a number of years and in more detail.
2) The measured response parameters should be selected carefully and broadly.
3) More ecosystem functions than just aboveground productivity should be followed.

4) Nutrient availability seems to be a key parameter in community assembly/ influencing the strength of priority effects. More experiments on harsher abiotic conditions at various different time scales should be done.
5) When investigating priority effects in community assembly also focusing on plant functional groups, especially legumes.
6) If in follow-up experiments the functional composition of the mixture were found to be more important than the species richness, one could perhaps use priority effects of initial sowing composition to direct the functional composition of the community as well as total aboveground biomass and cover.

The positive priority effect of the plant functional group of legumes on productivity found in the field, could be also confirmed in a pot experiment using more nutrient-rich potting soil and tested the effect of timing of arrival of functionally different species (priority effect 2), sowing density and sowing interval (simulation of dispersal frequency of natural assemblages) (*Manuscript 3*/ von Gillhaussen *et al.* 2014). In terms of productivity and functional group composition the simultaneously sown controls and the legume first treatment (priority effect 2) developed similarly. Which also underlies the importance of legumes species as keystone species for facilitation or complementary effects in species-rich grasslands. Legumes seem to be better able to get a head-start and grow quickly, compete well and allow facilitation for neighbors (hence a combination of asymmetric competition but also functional complementarity allowing for good community and population performance when legumes arrived early). Possible mechanisms behind priority effects are asymmetric competition (niche preemption), functional complementarity (changes in root traits) and plant soil-feedback (niche modification) (Grman & Suding 2010, Fukami 2015, Temperton *et al.* 2016). Plant soil-feedback could be negative and positive. One example of positive plant soil-feedback in relation to priority effects is the nitrogen facilitation between legumes and neighbors, because of changing the abiotic filter of the community by introducing extra nitrogen into soils either via nitrogen sparing or transfer. N sparing is if the legume species use lesser resources from soil nitrogen and N transfer can happen via decomposition, exudation or direct transfer via mycorrhizae so that extra legume nitrogen is released in the soil.

In general it could be shown that the priority effect of different order of arrival of plant functional groups was stronger than the sowing interval or sowing density effect.

This study gives us further direction in:

1) Testing this priority effect and positive legume influence also on different nutrient supplies like Kardol *et al.* (2013) to get general conclusions for influencing the development of plant communities via priority effects and their potential to create alternative stable states within plant communities.
2) Testing this greenhouse experiment also on field conditions, here it is important to test different functional groups and also different species within this group, different density and different sowing intervals and different nutrient supply levels as well as different time starting points and sites. In the meantime, field experiments to test priority effect 2 are done see also von Gillhaussen (2015), Weidlich *et al.* (2017).

Community assembly is affected by inhibitive and facilitative interactions (positive legume effects) between the resident and the arriving species, which can be seen in interspecific differences in mean trait values. But it is also important how the arriving species can react on this selection or filtering effect the resident species produced. The intraspecific trait variation depends on the individual species and can change community structure (see Figure 4). Trait variation plays an important role in trait-based environmental filtering one of the key processes implicated in plant community assembly (Siefert 2014). Priority effects interact with phenotypic plasticity and if we now know, that it is important who comes first and which function respectively which traits does it have how does it influence the traits variation of other species to fit in the filter or even how are species interact with different soil-conditions, how is the answer in traits on different neighbors, how they can react on different starting conditions. Important to take plasticity of traits of interacting species into account and analyzed this on long term. Which traits are relevant for species interaction (niche modification).

The analysis of the intraspecific trait variation of the target forb species *Plantago lanceolata* across two different soil nutrient levels and in interaction with other plant species in community assembly (*Manuscript 4*/Plückers *et al.* submitted soon) showed us again that soil nutrient availability was the main driving factor and that it also depend on the trait one focused on. Intriguingly, in the mesocosms the traits converged over time when comparing between the two soil nutrient treatments. In the field experiment soil nutrient availability had less influence on trait variation. This showed that the multivariate factors (heterogeneity of resource availability, competition, herbivory, weather), which act simultaneously on the species plant trait in the field, led to a more conservative and stable trait variation and more trait convergence, as well as especially legume neighborhood (again) played a role in creating

a positive facilitation and complementarity effects, but it does also depend on which factor and which trait you look at, because traits are different affected by abiotic and biotic conditions. Traits varied more strongly under the more-controlled mesocosm conditions than in the field. Soil fertility and especially non-optimal abiotic conditions led to higher plasticity under the more-controlled mesocosm conditions. On the one hand, it was found that plants were able to change their phenotype in response to environmental change. Indeed, it is often assumed that phenotypic plasticity has evolved again and again as an adaptation to environmental heterogeneity. On the other hand, many phenotypic responses to stressful environments may just be the consequence of passive reductions in growth due to resource limitation. Van Kleunen & Fischer (2005) stated that active and passive plastic responses of plants may act at the same time. Thus, our observed phenotypic responses to the environment may be the net result of both passive responses as a consequence of resource limitation and active responses as a consequence of changes in allocation. This is a hypothesis however, and needs further testing. Further studies should investigate whether these findings (influence of legumes stronger under field conditions rather than under more controlled conditions/under similar soil treatments, it was found quite specific responses in trait variation for field and also for controlled conditions in the mesocosms) hold true for other herbaceous species, as this could have important implications for interpolating between lab and field studies or across environmental gradients since translation of knowledge is not necessarily straightforward (Poorter *et al.* 2016).

This study gives further direction in:

1) The latter advocate taking the approach we follow in this study, which is to compare plant performance across similar abiotic/soil conditions and in the lab experiments to try to simulate conditions found in the field.
2) Other herbaceous species should be tested. The measured traits should be selected carefully, because intraspecific variation varied among traits (see also Siefert 2014).
3) Intraspecific trait variation should also be investigated in community assembly and priority effect experiments.

Whether one finds a priority effect or not and how stable it is may depend on which parameters one measures. The results of this thesis show us the importance of also focusing on plant functional groups, especially legumes in interaction with other groups, when investigating priority effects in community assembly. Furthermore nutrient availability had an abiding influence on the drivers on community assembly. In future we need general more

knowledge of the mechanisms and prevalence, relevance (how strong are effect sizes) and stability of priority effects (stable states or transient states), so that we can then hopefully steer communities in desired directions in terms of ecosystem functions, e.g. aiming for higher hay biomass, more carbon storage or other ecosystem services.

Increasingly the relative importance of historical contingency, including priority effects, for how communities function and how diverse they are is being acknowledged. In the past, the stochastic role of history in communities was generally ignored, either because was considered unimportant or it was too hard to study (Fukami 2015). Recent historical contingency research is underlining however, that history can be a defining factor in how communities develop over time (Brudvig 2011, Grman *et al.* 2013, Stuble *et al.* 2017, Weidlich *et al.* 2018). Future research should include these new findings and consider the relative strength of factors such as landscape configuration, soil conditions, management as well as historical contingency.

4 List of manuscripts and declaration of own contribution to each paper

Concept = Idea for the study and development of experimental design

Data acquisition = taking care of ongoing experimental plots and microcosms, being responsible for organization and execution of data acquisition and doing the measurements together with the help of interns

Data analysis = translation of raw data in digital tables, statistical analysis of data and their illustration

Literature research = acquisition of background information for introduction and discussion

Writing = writing the manuscript

Editing = General editing for the final version, including providing comments of co-authors and interns on the storyline and the scope

Manuscript 1

Title: Moving Towards Measuring Multifunctionality in Ecosystems: FieldScreen – A Mobile Positioning System for Non-Invasive Measurement of Plant Traits in Field Experiments

Authors: Christine Plückers, Vicky M. Temperton, André Erler, Alexander Putz, Hanno Scharr, and Uwe Rascher (Jülich)

Status: printed

Journal: Nova Acta Leopoldina NF *114*, Nr. 391, 221–237 (2013)

Own contribution:

Concept: 10%

Data acquisition: 95%

Data analysis: 95%

Literatur research: 70%

Writing: 80%

Editing: 80%

Manuscript 2

Title: Sowing different mixtures in dry acidic grassland produced priority effects of varying strength.

Authors: Christine Plückers, Uwe Rascher, Hanno Scharr, Philipp von Gillhaussen, Carl Beierkuhnlein, Vicky M. Temperton

Status: printed

Journal: Acta Oecologica-International Journal of Ecology, 53, 110-116. 2013 DOI: 10.1016/j.actao.2013.09.004

Own contribution:

Concept: 0%

Data acquisition: 95%

Data analyses: 95%

Literatur research: 70%

Writing: 75%

Editing: 70%

Manuscript 3

Title: Priority Effects of Time of Arrival of Plant Functional Groups Override Sowing Interval or Density Effects: A Grassland Experiment.

Authors: Philipp von Gillhaussen, Uwe Rascher, Nicolai D. Jablonowski, Christine Plückers, Carl Beierkuhnlein, Vicky M. Temperton

Status: printed

Journal: PLoS ONE, 9, e86906, 2014, DOI: 10.1371/journal.pone.0086906

Own contribution:

Concept: 0%

Data acquisition: 25%

Data analyses: 10%

Literatur research: 0%

Writing: 10%

Editing: 15%

(This article is also part of the dissertation of Philipp von Gillhaussen)

Manuscript 4

Title: Intraspecific phenotypic trait variation in *Plantago lanceolata* in field and mesocosm biodiversity experiments

Authors: Christine Plückers, Carl Beierkuhnlein, Vicky M. Temperton

Status: will be submitted

Own contribution:

Concept: 80%

Data acquisition: 90%

Data analyses: 100%

Literatur research: 90%

Writing: 90%

Editing: 80%

5 Conferences and trainings

Conference	When	Where	Own contribution	Title
GFÖ, 39st annual meeting "dimensions of ecology from global change to molecular ecology"	September 2009	Bayreuth, Germany	Poster	Following plant community assembly and plant phenotypic plasticity in different grassland habitats using traditional ecological and non-invasive high resolution census methods (FieldScreen)
10 Jahre BIOLOG-Forschung, Biodiversitätsforschung-Meilenstein zur Nachhaltigkeit, Wissenschaft und Praxis im Gespräch	March 2010	Berlin, Germany	-	Kongress
EGF, 23rd General meeting "Grassland in a Changing World"	August/ September 2010	Kiel, Germany	Poster	Following plant community assembly and plant phenotypic plasticity in different grassland habitats using traditional ecological and non-invasive high resolution census methods
Workshop SALVERE Projekt "Artenreiche Grünlandgesellschaften"	May 2011	Bernburg, Germany	Poster	Priority effects of initial sowing diversity on plant community assembly and productivity in dry grassland habitats
GFÖ, 41st annual meeting "Ecological functions, patterns, processes"	September 2011	Oldenburg, Germany	15' presentation	A novel non- invasive method in grassland assembly: using the *FIELDSCREEN* to follow changes over time
2nd International plant Phenotyping symposium	September 2011	Juelich, Germany	Poster (Vertreten durch Vicky Temperton)	Field phenotyping: a novel non- invasive method in grassland assembly
Institute seminar IBG-2	October	Juelich,	15'	Plant community

	2009	Germany	presentation	assembly in different grassland habitats- Comparing traditional ecological and FieldScreen phenotyping methods
Phytobilanz IBG-2	February 2010	Juelich, Germany	Poster	Following plant community assembly and plant phenotypic plasticity in different grassland habitats using traditional ecological and non-invasive high resolution census methods (FieldScreen)
Phytobilanz IBG-2	April 2012	Klein Altendorf, Germany	Poster 25' Presentation	Moving towards measuring multifunctionality in ecosystems: FieldScreen - A mobile positioning system for non-invasive measurement of plant traits in field experiments Assembly in grassland
Institute seminar IBG-2	April 2012	Juelich, Germany	15' presentation	My stay in the lab of Professor James Bullock, Centre for Ecology and Hydrology, England

Training	When	Where	Agency
Data analysis tools in ecology	February 2011	Coimbra, Portugal	Highland Statistics LtD, UK; IMAR
Effective oral presentation course	November 2011	Juelich, Germany	Principiae, Structuring thoughts
Stay abroad in another lab, for writing the manuscript and learning statistical analysis with R	January to March 2012	Wallingford, England	Centre for ecology and hydrology, Prof. James Bullock, Markus Wagner
Scientific writing course	March to June 2012	Juelich, Germany	Forschungszentrum Jülich GmbH, IBG-2 Vicky Temperton

6 References in introduction and conclusion

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7 Manuscripts

Manuscript 1

Moving Towards Measuring Multifunctionality in Ecosystems: FieldScreen – A Mobile Positioning System for Non-Invasive Measurement of Plant Traits in Field Experiments

Christine Plückers, Vicky M. Temperton, André Erler, Alexander Putz, Hanno Scharr, and Uwe Rascher

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Christine Plückers, Vicky M. Temperton, André Erler, Alexander Putz, Hanno Scharr, and Uwe Rascher (Jülich)

With 4 Figures and 1 Table

Abstract

In the face of rapidly declining diversity interest in how plant diversity and ecosystem functioning interrelate and how this relationship may differ across various systems is high. We know that grasslands with more species and functional traits interacting can positively affect ecosystem functioning such as productivity or nutrient cycling. These findings usually relate to highly managed experiments, however, and we still know little of how diversity and ecosystem function relate in more natural systems subjected to invasion. Latest findings also point to the need to focus on more than a few ecosystem functions (multifunctionality), and hence also a suite of traits of species, at the same time to better understand how diversity and ecosystem properties are connected. Ecosystems are subjected to dynamic changes at many different spatial and time scales. There are short-term variabilities, rhythms over days or years, and changes and interaction happening on longer time scales. These dynamic changes in nature can lead to alteration of ecosystem functions over time. To describe these changes and the multifunctionality of ecosystems, spatial and temporal analyses at various scales are essential and new approaches are necessary to complement traditional ecological measurements.

Here we present a combined approach linking community assembly and physiological research with an automated non-invasive positioning system for measuring multiple traits of vegetation in the field. The "FieldScreen" is set up over the "Habitat Garden" Experiment, a grassland assembly experiment. The FieldScreen can accurately position a set of sensors enabling automated measurements of the plants and soil surface below by means of high-resolution photos, hyper-spectral reflectance or sun-induced fluorescence measurements. The Habitat Garden Experiment addresses how priority effects of species that arrive first in a system may affect both productivity and diversity (assembly) over time.

The first 3 years of observation showed that with the FieldScreen it is possible to non-invasively follow changes of species turnover and selected plant traits over time. With the photos taken with a camera mounted on the FieldScreen trolley we can clearly distinguish that sowing initially different diversity levels has an abiding influence on the further development of the plant communities, the spatial spread of species and the overall vegetation cover. These time series have the potential to address research questions on the dynamic nature of ecosystem functioning. This could include measuring several traits of plants at the same time and hence helping to address the need to measure multifunctionality in natural systems if we are to better understand how diversity and ecosystem functioning are linked in natural systems subjected to many disturbances and drivers.

Zusammenfassung

Angesichts des weltweiten Artenverlustes ist das Interesse, die Zusammenhänge zwischen Pflanzenvielfalt und den Funktionen von Ökosystemen und deren Veränderung zu verstehen, hoch. Graslandgesellschaften mit einer hohen Artenvielfalt und dazugehörigen funktionellen Merkmalen haben positive Auswirkungen auf verschiedene Ökosystemfunktionen wie z. B. Produktivität und Stickstoffhaushalt. Diese Zusammenhänge wurden jedoch in kontrollierten Experimenten gefunden, und es ist bisher noch unklar, inwieweit Zusammenhänge zwischen Artenvielfalt und Ökosystemfunktion in natürlichen Systemen bestehen, die durch Einwanderung neuer Arten charakterisiert sind. Um die Zusammenhänge zwischen Diversität und Ökosystemeigenschaften besser zu verstehen, ist es nötig,

Beobachtungen nicht nur auf ausgewählte Ökosystemfunktionen zu fokussieren, sondern ein Ökosystem als Gesamtgefüge zu betrachten und gleichzeitig die Merkmale von Arten einzubeziehen. Ökosysteme stehen ständig in Wechselbeziehung mit anderen Organismen und der Umwelt und verändern sich dynamisch auf unterschiedlichen Zeitskalen. Diese Veränderungen können kurzzeitliche Änderungen sein oder auch Rhythmen über Tage, Jahre oder sogar auch Veränderungen und Interaktionen auf längeren Zeitskalen. Diese dynamischen Änderungen in der Natur (z. B. Veränderung von Artenzusammensetzungen und Einwanderung von Arten) können zu Veränderungen in den Funktionen der Ökosysteme über die Zeit führen. Um Aussagen über die Änderungen von Ökosystemen machen zu können, bedarf es zeitlich und räumlich entsprechend aufgelöster Untersuchungen mittels neuer technischer Ansätze.

Wir präsentieren hier einen experimentellen Ansatz, bei dem Untersuchungen zu Pflanzengesellschaftsentwicklungen und physiologische Messungen verknüpft werden. Ein neu entwickeltes mobiles Positionierungssystem (‚FieldScreen') wurde über dem ‚Habitat Garten', einem Experiment zur Untersuchung der Graslandgesellschaftsentwicklung, errichtet. Der FieldScreen verfügt über eine bewegliche Traverse, mit der verschiedene Sensoren automatisch positioniert und zeitlich wiederholte Messungen durchgeführt werden können (zurzeit Kameraaufnahmen und Messungen der hyperspektralen Reflexion und sonneninduzierten Fluoreszenz).

In den ersten drei Jahren konnte gezeigt werden, dass es möglich ist, mit Hilfe des FieldScreen nicht-invasiv detaillierte Veränderungen des Artenumsatzes und ausgewählter Pflanzenmerkmale über die Zeit aufzunehmen. Die anfänglich unterschiedliche Artenzusammensetzungen der trockenen Grasgesellschaften hatten einen deutlichen Einfluss auf die weitere Entwicklung der Pflanzengesellschaften, und Unterschiede in der räumlichen Ausbreitung von Arten und des Bedeckungsgrades wurden sichtbar. Mit dem FieldScreen ist es nun möglich, ökologische und physiologische Informationen kontinuierlich über eine gesamte Vegetationsperiode zu erhalten. Solche Zeitserien haben das Potenzial, Informationen über Entwicklungsprozesse von Ökosystemen in ihrer Gesamtheit zu liefern und damit die Gesamtfunktionen in natürlichen Systemen zu beschreiben.

1. Introduction

Global change, including not only climate change but also the consequent alteration of ecosystems due to biodiversity loss, land use change, and invasion by exotic species, for example, are major challenges of our time (Chapin et al. 2000). Society and politicians are requesting practical solutions to the multiple current strains on ecosystems (Beck 1993). To get a grasp on how global change is affecting how ecosystems work and provide ecosystem services (Diaz et al. 2006, 2007), environmental and ecological science have to rise to the challenge to understand already highly complex and dynamic ecological systems within a changing environment (Harris et al. 2006). To follow changes in vegetation we will need a whole suite of skills ranging from traditional ecological expertise (knowledge of species and their interactions), through better global monitoring of various habitat types to high-tech measurement of dynamic changes at various scales - both temporal and spatial (e.g. deriving physiologically or ecologically significant information on plant performance through remote sensing of vegetation; Rascher and Pieruschka 2008, Ustin and Gamon 2010). Information on plant performance derived at one scale or under one set of controlled conditions, needs to be now linked and integrated with plant performance at larger scales and under more natural conditions (Körner 1995).

In recent decades a high decline of biodiversity has been observed worldwide (Loreau et al. 2001, Butchart et al. 2010, Isbell et al. 2011), which can have a negative impact on ecosystem functioning and services (Schulze and Mooney 1993, Balvanera et al. 2006). Major direct drivers of biodiversity loss are climate change, invasion of exotic, non-native species, habitat destruction and degradation or land use changes at local and regional scale (De Meester et al. 2010). Land use changes, specifically the conversion of natural ecosystems into agro-ecosystems have led to changes in the species composition and to a decrease of biodiversity (van der Putten et al. 2000).

Semi-natural grasslands are the vegetation type with the highest diversity of species per m^2 world-wide (Willems et al. 1993). They form a key part of the European culturally-formed landscape and are maintained either by grazing or mowing. Due to both intensification of land use, and land abandonment, the area covered by semi-natural grasslands has shrunk considerably over the past century such that many native grassland species are now endangered (Rusina and Kiehl 2010, Kirmer et al. 2011). As well as preserving existing species-rich grassland habitats, recreating grasslands (restoring) is becoming a common tool to counteract biodiversity loss, either on ex-arable land (Bullock et al. 2007) or on marginal land (land not fit for intensive agriculture due to poor nutrients in soils, e.g. former mining sites [Baasch et al. 2012]).

In ecological science many biodiversity experiments, where for example plant diversity is manipulated and its effect on ecosystem properties such as productivity or nutrient cycling is followed, have shown that both species richness and functional diversity (i.e. the functional traits of the species in a system) can have positive effects on ecosystem functioning, especially in grassland systems (Schläpfer and Schmid 1999, Schläpfer et al. 1999, Hooper et al. 2005, Balvanera et al. 2006, Díaz et al. 2006). More diverse grasslands in biodiversity-ecosystem experiments, in which plots were sown with highly diverse plant seed mixtures, were found to be more productive and to take up available resources more efficiently than less diverse mixtures, and this positive diversity effect even increased with time (Marquard et al. 2009). In particular, very recent biodiversity research has shown that over time in a grassland (Allan et al. 2011) the species interacting most positively and hence driving ecosystem properties (such as productivity) change over time, such that a whole suite of species are necessary to maintain function over a longer time span, refuting early possible implications of such diversity experiments that only a small number of species may be necessary to maintain certain ecosystem functions such as nutrient cycling.

It is now becoming clear that if more functions of an ecosystem are considered, then more species or functional traits are needed to maintain those functions (so-called multifunctionality), particularly in face of climate change (Zavaleta et al. 2010). To focus more on studies of multifunctionality in the future will require either more researchers working together in one system, looking at many of its different functions, or the use of technology that enables simultaneous measurement of a suite of properties of an ecosystem.

Biodiversity Ecosystem functioning experiments are somewhat unnatural, however, in that species are chosen at random from a total species pool, and species levels are maintained by weeding (i.e. are "closed" systems). This poses the question therefore as to how important such positive diversity effects may be in more natural systems where there is immigration of species into and out of the system ("open" systems) (Srivastava et al. 2004).

The positive relationship found in biodiversity experiments between diversity and functioning of an ecosystem should motivate restoration practitioners to include a larger number of species during grassland restoration plantings or sowing (see successful examples in Bullock et al. 2001, 2007). But it is not yet generally clear whether this positive relationship between diversity and functioning of ecosystems also holds true in restoration of different habitat types and over long time, because this relationship has been developed through several tests involving short-term and highly controlled experiments (Doherty et al. 2011). There is still no clear picture of how many species or how many species with specific functional traits are needed in an ecosystem to maintain specific functions (Isbell et al. 2011). We need to know more about how initial condition (including e.g. the diversity of plant species

sown, so-called priority effects [Facelli and Facelli 1993, Fukami et al. 2005, Bullock et al. 2001, Grman and Suding 2009]) influence the further development of diversity and ecosystem functioning within natural and semi-natural systems such as grasslands. In short we need to investigate how dynamic community assembly (vegetation development) interacts with biodiversity effects.

Only a few research groups have actively tested whether positive biodiversity effects found in ecosystem-functioning experiments also occur in systems with natural assembly as well as in degraded systems or in ecosystems with extreme or highly fluctuating environments like dry lands (van der Putten 2000, Bezemer and van der Putten 2007, Lepŝ et al. 2007, Tischew and Kirmer 2007, Bullock et al. 2007, Kirmer et al. 2008, Baasch et al. 2009, Doherty et al. 2011).

Ecological systems going through natural assembly, and especially grasslands, are highly dynamic. No two years are the same in terms of plant species composition due to weather fluctuations and other random factors. Changes occur not only at different spatial but also at different time scales, ranging from minutes, through days, months, years, to changes and interactions needing more time (evolutionary processes) (von der Wiesche and Werner 1998). Research questions such as effects of biodiversity loss or differences in assembly and further development due to starting diversity are scale-dependent and outcomes of measurements will be highly spatially and temporally dependent.

Obtaining a relatively complete description of the spatial and temporal changes and interactions in an ecosystem requires very high costs with a high effort (costs, man power and time) and is in general not feasible. Therefore, researchers have to focus on a few aspects they want to analyze in their experiments, because of such limitations. This can lead to a loss of important information for understanding restoration and assembly under natural conditions. For this reason, we need to test the potential for using measurement of a number of plant or canopy traits at the same time (knowing that traits and species relate directly to ecosystem function, see above), and at wide range of time spans or scales than has been possible using usual workload.

We test new approaches using technology specifically developed to address research questions on multifunctionality, to complement ecological expertise on the ground, to assess changes in ecosystems over space and time and how they relate to the functioning of these systems. One new approach is a Mobile Field Positioning System called "FieldScreen", which accurately positions a sensor and enables automated and repeated non-invasive measurements of the plants and soil surface in the "Habitat Garden" (Fig. 1*A*). A sensor platform taking measurements for example involving high resolution photos, hyper-spectral reflectance or sun-induced fluorescence. In this manuscript we present the strengths and possibilities of this new approach in a grassland assembly experiment. We use the FieldScreen to follow changes both spatially and over time in different grassland habitats and their development over the course of several years. We want to test or to see what kind of ecological or physiological traits can be followed using the FieldScreen at community and species-level. We describe the experimental design, explain the construction and measurement concept of the Habitat Garden and the FieldScreen and present the first results of the FieldScreen from the first four years measurements of seasonal dynamics of vegetation development (assembly) in the experiment.

2. The "Habitat Garden": A Community Assembly Field Experiment to Quantify How Diversity and Ecosystem Function is Affected by Priority Effects

In December 2007 we started a grassland assembly experiment, the Habitat Garden, with two different grassland habitats (dry and mesic grassland). The dry grassland plots were initially sown with two different diversity levels (2 grass and 25 forbs *versus* 7 grass and 32 forbs) and the mesic plots were sown with the same diversity of species at the start. The experiment was designed to quantify changes in early successional grassland systems over time (e.g. diversity, productivity, spatial spread of species, phenology of plant species, specifical traits of species) depending on the different starting diversity (priority effects).

The ecological field plots were set up on an ex-arable soil in the area of the Forschungszentrum Jülich, located in West Germany (6° 22'0"E, 50° 56'0"N). 12 plots were prepared, each plot being 2 × 2 m in size and separated from each other by 1 m rows. The 12 plots were set-up as randomized and three different treatments were selected: a mesic grassland (M), a dry acidic grassland with medium species diversity (S2) and a dry acidic grassland with high species diversity (S7) (Fig. 1*B*). For each plot the original soil was removed to a depth of 40 cm, a geomembrane permeable to water and nutrient laid to avoid germination of seeds from the origin soil. For the mesic grasslands an ex-arable soil type of the region Heinsberg, Germany (Geilenkirchen 6° 7'0"E, 50° 58'0"N) was selected, for the dry grasslands washed sand (Quarzwerke Witterschlick GmbH, grain size 0.7–1.4 mm) mixed with 10 % potting soil (Einheitserde Werkverband e. V.) was used.

The plots were hand-sown in the first year only, with seed mixes from Rieger Hofmann GmbH, a company specializing in native grassland seed mixes of local provenance. The seeding rate for each plot was 4 g/ m^2. The mesic grassland with moderate soil nutrients availability (M in Fig. 1*B*) was sown with a seed mix containing 11 grass species and 23 forbs (including 2 legumes) (Tab. 1*A* and *B*) and removal experiments (removing specific species or functional groups of plants) were performed at later dates providing ecological experimental treatments (data not shown). The dry acidic grassland communities were established on sand mixed with one tenth potting soil with very low nutrient and water availability (S in Fig. 1*B*) and was sown with two biodiversity levels: a medium diversity seed mixture with either 2 starting grass species and 25 forb species (including 1 legume) (S2 in Fig. 1*B*) and a high diversity seed mixture with either 7 starting grass species and 32 forb species (including 4 legumes) (S7 in Fig. 1*B*) (Tab. 1*A* and *B*). One quarter of every plot was not sown and kept as control subplot (upper right corner of each plot in Fig. 1*B*). The whole area was fenced to reduce confounding factors such as grazing by deer or wild boar.

During the experiment invasion of new species could occur in three ways (in addition to establishment from the sown seeds): from the seed bank in soil, from neighboring plots or from vegetation outside of the Habitat Garden. The sites were not fertilized, but they were mown ones or twice a year depending on the grassland habitats (dry grasslands once a year in August, mesic grasslands twice a year in early June and late August according to typical mowing regimes for such grasslands in Central Europe). In the first year (2008) there was only one late hay cut in October for the mesic grasslands. In the next years there were two hay cuts in June and in August for the mesic grasslands and only one hay cut every year in the end of July beginning of August for the dry grasslands at the vegetation peak.

Since 2007 establishment of species and the dynamics of restoration was continuously monitored. Every year before mowing vegetation was assessed for the presence and absence

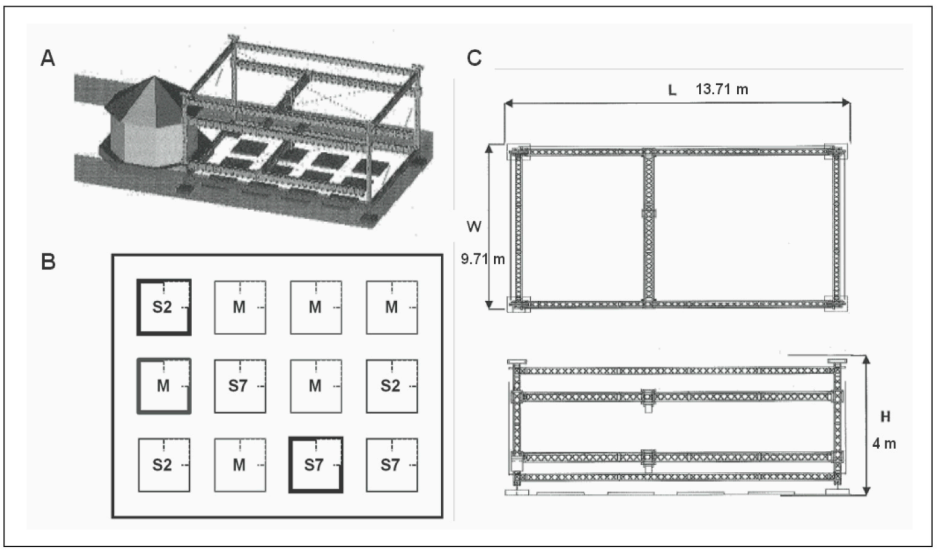

Fig. 1 (*A*) Schematic view of the FieldScreen set up over the plots in the Habitat Garden (© Manual FieldScreen, Visser International trade and engineering B. V. ['s-Gravendeel, Netherlands]). (*B*) Layout of the field experiment showing the plots sown in December 2007 with mesic grassland species (M) or with dry grassland species (S), S2 is the species mixtures with 2 starting grass species and 25 forb species (including 1 legume) sown, S7 with 7 starting grass species and 32 forb species (including 4 legumes) sown. Three plots (one plot of each regime, identified by bold outline), were subject to further analysis (Fig. 2, 3, 4). In one corner of every plot the dotted line shows the control region where no seeds were sown. (*C*) Frame of the FieldScreen with the maximum size of a height of 4 m a width of 9.71 m, and a length of 13.71 m (© Manual FieldScreen, Visser International trade and engineering B. V. ['s-Gravendeel, Netherlands]).

of each species. Additionally the cover of every species was visually estimated using a decimal scale (Londo 1976). In the first year (2008) two vegetation assessments for the mesic grassland and one for the dry grassland were done even though there was only one hay cut for the mesic grassland and non for the dry grassland. To calculate the invasion pressure the species number and their cover at the plot border was also estimated separately. Aboveground biomass production (dry matter yield) was measured in two 0.1 m^2 quadrates in every plot (one in the control area and one randomly in the plot) before mowing. Plant material was cut 3 cm above the soil surface and dry weight was taken (dried biomass is stored for potential later analyses). In 2010 and 2011 biomass was sorted according to three functional groups: legumes, forbs and grass species. In June and August 2011 the leaf area index (LAI) was measured in all grassland plots using a LAI-meter of Licor (LAI-2000, Plant canopy analyzer, Licor Biosciences, Lincoln, USA). Furthermore, every year soil samples were taken from the top soil layer (0–15 cm) and analyzed for pH, total P content, mineralized soil N, and total C and N content.

Since 2010 various functional traits have been recorded for *Lotus corniculatus*, *Plantago lanceolata*, *Trifolium* spec., *Ranunculus* spec. and *Hypochaeris radicata*. Photosynthetic light response curves were measured with a Fluorescence Yield Analyzer Mini-PAM (Heinz Walz GmbH, Effeltrich, Germany). Chlorophyll content for samples from 2–5 individual

plants of each species was analyzed by an extraction method and a spectro-photometrical analysis (LICHTENTHALER 1987, LICHTENTHALER und BUSCHMANN 2001). For a sample of 3–5 leaves from each species, the area was estimated with a leaf area meter (LI-3100C, Licor Biosciences, Lincoln, USA) and fresh and dry weight was taken to calculate leaf water content and specific leaf area. Nitrogen and carbon content was determined from dried leaf samples. Results on dynamic changes of functional traits during establishment are currently being analyzed and will be reported elsewhere.

Tab. 1 List of species sown in the Habitat Garden in 2007, in the mesic grassland plots or in the dry grassland plots, S2 is the species mixtures with 2 starting grass species and 25 forb species (including 1 legume) sown, S7 with 7 starting grass species and 32 forb species (including 4 legumes) sown. A List of grass species. B List of forb species including legumes which are shown in bold font.

A	Species	Dry grassland		Mesic grassland
		S2	S7	
Grass species	*Agrostis capillaris*		×	
	Alopecurus pratensis			×
	Anthoxanthum odoratum			×
	Arrhenatherum elatius			×
	Bromus erectus			×
	Corynephorus canescens	×	×	
	Cynosurus cristatus			×
	Dactylis glomerata			×
	Deschampsia flexuosa		×	
	Festuca guestfalica	×	×	
	Festuca nigrescens		×	×
	Festuca pratensis			×
	Helictotrichon pubescens			×
	Luzula campestris		×	
	Poa compressa		×	
	Poa pratensis			×
	Trisetum flavescens			×
B	Species	Dry grassland		Mesic grassland
		S2	S7	
Forb species	*Achillea millefolium*	×	×	×
	Anchusa officinalis	×		
	Anthemis arvensis		×	
	Anthriscus sylvestris			×
	Armeria maritime/elongata	×	×	
	Artemisa campestris	×		
	Berteroa incana		×	
	Calluna vulgaris	×		
	Campanula patula			×
	Campanula rotundifolia	×	×	
	Carum carvi			×
	Centaurea jacea			×
	Chrysanthemum segetum		×	

B	Species	Dry grassland		Mesic grassland
		S2	S7	
	Crepis biennis			×
	Daucus carota	×	×	×
	Dianthus carthusianorum	×		
	Dianthus deltoides	×	×	
	Echium vulgare	×	×	
	Galium album	×		×
	Galium verum		×	
	Genista tinctoria		×	
	Hieracium pilosella	×	×	
	Hypericum perforatum	×	×	
	Hypochaeris radicata	×	×	
	Jasione montana	×	×	
	Knautia arvensis			×
	Leontodon autumnalis	×		
	Leontodon hispidus	×	×	×
	Leucanthemum ircutianum			×
	Linaria vulgaris		×	
	Lotus corniculatus		×	×
	Oenothera biennis	×		
	Papaver argemone		×	
	Papaver rhoeas			×
	Petrorhagia prolifera	×		
	Pimpinella major			×
	Plantago lanceolata		×	×
	Potentilla argentea	×		
	Prunella vulgaris		×	×
	Ranunculus acris			×
	Reseda lutea		×	
	Reseda luteola		×	
	Rumex acetosa			×
	Rumex acetosella		×	
	Salvia pratenis			×
	Sedum acre	×	×	
	Silene flos-cuculi			×
	Silene latifolia ssp alba		×	
	Silene viscaria		×	
	Silene vulgaris	×		×
	Spergula arvensis		×	
	Thymus pulegioides		×	
	Tragopogon pratensis			×
	Trifolium arvense	×	×	
	Trifolium campestre		×	
	Trifolium pratense			×
	Verbascum thapsus	×		
	Verbascum densiflorum	×		
	Viola tricolor		×	

3. The "FieldScreen": A New Method to Non-invasively Track and Quantify Functional and Structural Traits of Plants During Assembly.

The FieldScreen was installed over the Habitat Garden in 2009 to follow the development and dynamic changes in this grassland community assembly over time (years and also during the course of days). Conventional ecological assessments (see above) can only be taken at selected time points during the course of the year and thus may miss multifunctionality during assembly. The FieldScreen provides a new approach to provide temporally high resolution data during an ecologically highly dynamic process. With the FieldScreen we aim to non-invasively observe plots, to characterize dominance of species, and to quantify their functional and structural traits during this assembly process.

The FieldScreen is a mobile scanner transport system that was built by Visser International trade and engineering B.V ('s-Gravendeel, Netherlands) (Fig. 2*A*). The whole system consists of 42 parts, none of which is longer than 5 m and heavier than 50 kg. The whole FieldScreen weighs 400 kg. The major part of the frame consists of standard aluminum truss modules with minor modifications. The single parts are connected by a robust plugging system allowing a rapid and easy assembly. The FieldScreen can thus be assembled in half a day with 4–6 persons, allowing to set up the system in fields not accessible to heavy machines. However, a 400 V electric power supply needs to be available to power the electric drives. The frame and electric parts are completely weather-resistant. However, the traverse may not be operated during frost and snow. The FieldScreen spans a total length of 13.71 m and a width of 9.71 m (Fig. 1*C*). The height is adjustable up to 4 m. On top of the upper frame is a movable traverse (Fig. 2*A*) which can be moved and exactly positioned by a PLC (programmable logic control) using a laser positioning system as a positioning feedback. A traveling trolley carrying up to 50 kg of measurement equipment is driven on this traverse and can be moved and positioned along the traverse. Traverse and trolley (sensor platform) can be positioned with an absolute accuracy better than 1 cm by the laser positioning system and an arbitrary series of measurement positions can be programmed. The velocity of the traverse and the slide is 5 and 10 cm/ s, respectively.

In our case we have fixed the traverse at 4 m height and have installed two different sensors. First, we installed a conventional programmable digital camera (Imperx Inc., Boca Raton, USA, IPX-11M5-G: Imperx 11 Mega pixel/ Nikon AF Nikkor objective 50 mm 1:1.4D) with a water protective casing (Stemmer Imaging GmbH, Puchheim, Germany). This camera is a high resolution, industrial grade, fully programmable CCD camera. The camera provides a 4000 × 2672 pixel resolution and can deliver up to 5 frames per second at full resolution. By inspection of the photos we are able to visually identify and characterize single species within the plots, including time of flowering. Additionally, the spreading of vegetation and the invasion of species was followed. In the future we aim for quantifying total vegetation cover (2D) automatically by applying suitable texture classification and image segmentation algorithms.

Secondly, a hyperspectral measuring unit was developed and installed. The unit consists of two HR4000 spectrometers (Ocean optics, Dunedin, USA) having different spectral ranges (200–1100 nm / 670–860 nm) and resolution (0.1 nm and 0.01 nm), housed in a water-proof and temperature controlled box. One spectrometer is used for computation of vegetation indices, the other to estimate sun-induced fluorescence (Fs) using the atmospheric O_2-absorption bands (Rossini et al. 2010a, b). Light is collected by two fibre-optics one looking upwards for incoming radiation, one looking downwards for reflected radiation. The openings of the fibers are covered by a custom made cosine receptor that was adapted from Rossini et al. (2010a, b). Light of the fiber optics is divided by an optical multiplexer allowing simultane-

ous measurement from the two spectrometers (Meroni et al. 2010, Rossini et al. 2010a, b). An optical multiplexer can switch between channels measuring the light of both fibre-optics (looking upwards and downwards) and a blind channel for dark current measurements (Meroni et al. 2010, Rossini et al. 2010a, b). The data of the spectrometers are currently analyzed and will allow calculation of hyperspectral canopy reflectance, a variety of vegetation indices, and sun-induced fluorescence (not shown) (Malenovsky et al. 2010, Meroni et al. 2009, Rascher et al. 2011).

Automation of the positioning system and the coordinated measurement of all sensors are ensured by a dedicated software program (Erler 2011). This software synchronizes imaging and hyperspectral measurements with the automation software for the positioning of the FieldScreen. A small computer inside the hyperspectral measuring box communicates with the PLC and further controls the hardware that is needed for the measurements, e.g. cameras, two hyperspectral sensors and an optical multiplexer to switch between incoming and reflected light measurements. The software can calculate the sun-induced chlorophyll fluorescence at wavelength of absorption bands on-the-fly. One of three implemented Fraunhofer Line Discrimination approaches (namely the standard FLD method, the Maier-Method and the improved FLD method, Meroni et al. 2009) can be used.

Since the establishment in 2009, FieldScreen measurements of every plot were performed from March to November every year if the weather conditions were favorable (i.e. clear skies). In 2009 data were recorded on 10 measurements days, in 2010 17 measurement days, and in 2011 33 measurements days.

4. Following Visual Changes in the Development of the Different Grassland Habitats Over 4 Years

Seasonal changes of the grassland were observed with high resolution cameras over 4 years and characteristic differences between the two diversity treatments (testing priority effects) of the dry grassland and the mesic grassland are detectable (Fig. 2).

In Figure *2B–D* time series showing vegetation development between 2008 (photos taken by hand with a camera, since 2009 photos taken with a camera mounted on the trolley of the FieldScreen) and 2011 in three representative plots: a dry grassland plot with high species diversity (S7) (Fig. 1*B* plot marked bold outline), a dry grassland plot with medium species diversity (S2) (Fig. 1*B* plot marked bold outline) and a mesic grassland plot (M) (Fig. 1*B* plot marked bold outline).

By visually comparing the photos (Fig. 2*B–C*) it is clear that the S7 plot had a faster canopy closure and also a higher biomass production. Also differences in species presence between the two diversity treatments occurred and are visible in the photos (Fig. 2*B–C*). In the S7 plot the dominant species was the nitrogen fixing *Lotus corniculatus* (yellow flowers in Fig. 2*B*). In photo 4 of Figure 2*B* (07/2010) the pink flowers of *Dianthus deltoides*, a forb species are visible between the yellow flowers of *Lotus corniculatus.* In photo 5 of Figure 2*B* one year later (07/2011) a white flowering species has appeared, which was identified as *Daucus carota*, a tall forb species. All these were species that had been sown at the beginning of experiment. For comparison in the S2 plot also a yellow flowering species could be identified using the photos but the anatomy of the flower was different to *Lotus corniculatus*. This yellow species was *Hieracium pilosella*, another forb species. In photo 4 Figure 2*C* (07/2010)

Dianthus deltoides with the pink flowers is also clearly visible in the S2 plot. Furthermore, in photo 5 of Figure 2*C* (07/2011) a white flowering species has appeared, this is *Achillea millefolium* a forb species. Clearly visible in the free spaces of the plots is the grass *Corynephorus canescens*, sown at the beginning of experiment as well as the other dominant species.

Figure 2*D* shows a time series (2008–2011) of one mesic grassland plot. Directly in the first year a high canopy closure has existed, but over the next years biomass increased, as all species have grown more compactly. In the first two photos (05/2008 and 07/2008) there are visible additional species that were not sown, mainly species from the seed bank of the soil. Starting 2009 the sown species become observable. Comparing mesic and dry grassland plots, fewer flowering species were growing (depends on the sown mixtures), but all growing species had a higher biomass production and vegetation cover in all years. The dominant flowering species were *Leucanthemum ircutianum* and *Crepis biennis*. *Rumex acetosa* had a dominant ground cover because of its big leaves.

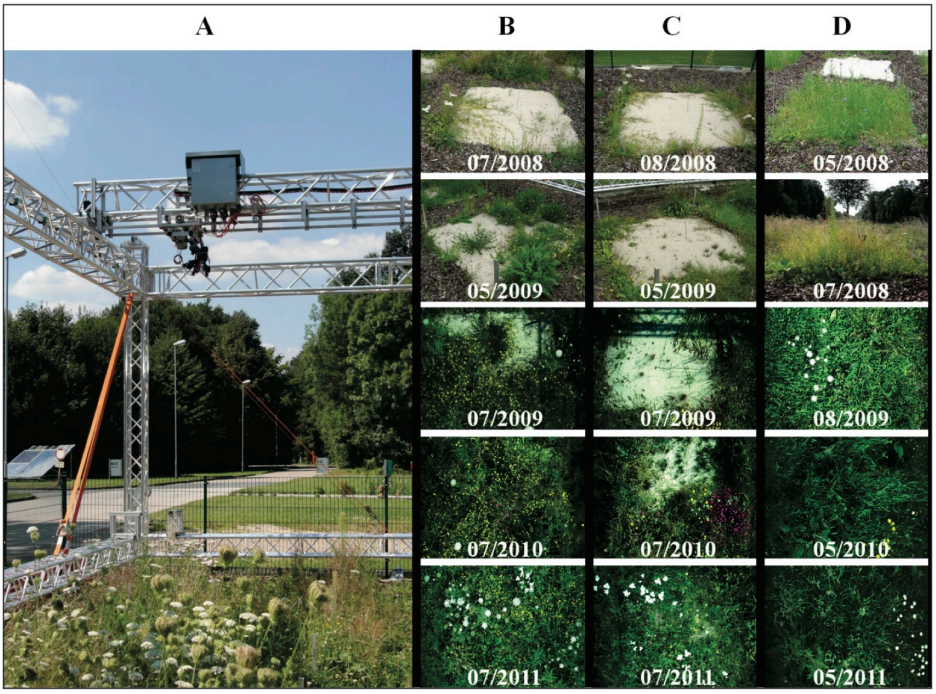

Fig. 2 (*A*) View of the FieldScreen set up over the Habitat Garden assembly experiment at Plant Sciences (IBG-2) Forschungszentrum Jülich. (*B*, *C*, *D*) time series showing vegetation development from 2008 over 4 years in three representative plots for the different grassland habitats. (*B*) Dry grassland sown with 7 starting grass species and 32 forb species (S7 plot marked bold in Fig. 1*B*) (*C*) Dry grassland sown with 2 starting grass species and 25 forb species (S2 plot marked bold in Fig. 1*B*) (*D*) Mesic grassland plots (M plot marked bold in Fig. 1*B*). The top two rows were taken by hand-held camera, whereas the last three rows of photos for each representative plot were taken using a camera mounted on the trolley on the FieldScreen and are currently being assessed for potential to follow phenology, invasion timing of specific species and canopy structure using image analysis tools.

In Figures 3 and 4 time series of both diversity levels within the dry grassland are shown in more detail, covering the dynamics of 3 seasonal cycles (2009–2011). In Figure 2 only one time point in the year is shown and one may assume that in 2009 the only dominant species in S7 plot was *Lotus corniculatus* (Fig. 2*B* 07/2009 and Fig. 3*A*). Looking in detail (Fig. 3) at the temporal changes it is clear that species dominance changed: early in vegetation period 2009 *Daucus carata* was growing strongly and became dominant (Fig. 3*B* and *C*), but after mowing *Lotus corniculatus* and *Plantago lanceolata* had the fastest regrowth (Fig. 3*D* and *E*). In 2010 *Daucus carota* was not dominant (Fig. 3*J*), but the cover of *Lotus corniculatus* remained high. *Dianthus deltoides* (Fig. 3*I*) was also growing strongly in 2010 and also more grass species appeared between the forbs (Fig. 3*J*). In Figure 3*N* and *O*, showing recovery after mowing, there was fast recovery of *Plantago lanceolata* and *Lotus corniculatus*. The first canopy closure occurred in July 2010 (Fig. 3*I*).

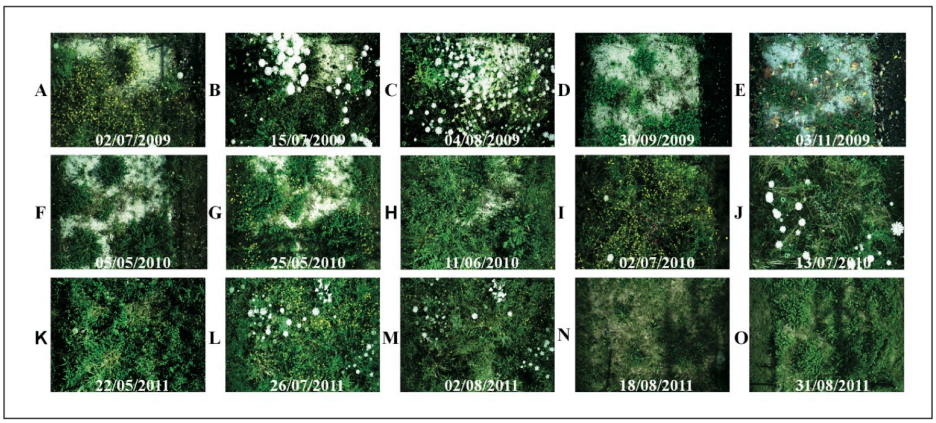

Fig. 3 A photographic time series over one example S7 dry grassland plot in more detail (7 starting grass species and 32 forb species sown, S7 plot marked bold in Fig. 1B). (*A–E*) July – November 2009, (*F–J*) May – July 2010, (*K–O*) May – End of August 2011.

For the S2 plot in Figure 4 the canopy closure has still not reached 100 % (*O*), and the dynamics of strong or dominant growing species is less compared to the S7 plot. By examining only one time point in the year (Fig. 2*C*) this interpretation is not possible. There, the dynamics of both systems look more similar and also the canopy closure (Fig. 2*C* 07/2011) seems to be 100 %. That this is not the fact is clear after mowing (Fig. 4*N* and *O*). But from all three years (Fig. 4) a higher canopy closure is visible since 2009. The less dynamics of dominant species is only visible through the detailed photo series (Fig. 4). The same dominant flowering species (Fig. 4*A*, *B*, *H* and *I*) *Dianthus deltoides* (pinc) and *Hieracium pilosella* (yellow) are also visible, as in the photos of Figure 2*C*, but because observation was frequent, we can be sure that that the cover for each species was always higher in 2010 than in 2009. Also *Achillea millefolium* (white) can be identified as more dominant in 2011 (Fig. 4*L* and *M*) compared to 2009 (Fig. 4*A–C*). For the year 2010 (Fig. 4*J*) also grass species can be identified, only by frequent observation, not be seen on one time point in the year (Fig. 2*C* 07/2010). The species *Cirsium arvense*, which had not been sown at the

beginning of the experiment, invaded both grassland systems, and more so in 2011 (Fig. 3 and Fig. 4*K–O*) than in 2010 (Fig. 3 and Fig. 4*F–J*).

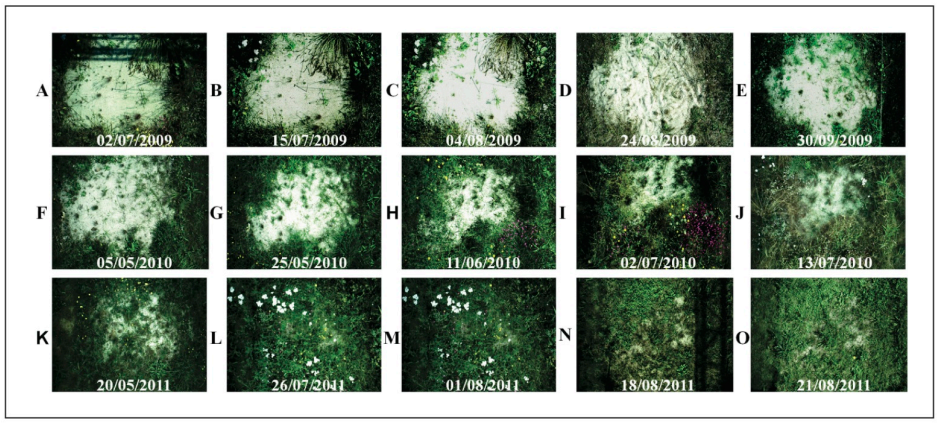

Fig. 4 A photographic time series over one example S2 dry grassland plot in more detail (2 starting grass species and 25 forb species sown, S2 plot marked bold in Fig. 1*B*). (*A–E*) July – November 2009, (*F–J*) May – July 2010, (*K–O*) May – August 2011.

5. Potential of the FieldScreen of Physiology and Ecology

The FieldScreen can follow changes in the development of plant communities over time. In the process it is possible to measure species and also vegetation traits with different kind of sensors. Using the photographs it is possible to gain information on: flowering phenology, species presence and absence, dominant species, vegetation spread and invasion of species and total vegetation cover (2D). In the future the data of the spectrometers will allow calculation of hyperspectral canopy reflectance, a variety of vegetation indices, and sun-induced fluorescence.

6. Conclusion

We have shown, that photos taken by the FieldScreen allow identification of single species, especially dominant species and plants traits, prevalent the phenology (flowering time) over time, but it was not possible to identify every single species or the cover of every species in this highly diverse system like conventional ecological assessments can. The total 2 dimension canopy cover can be estimated using the photos, but not the 3 dimensional cover. Nevertheless, for research on open ecological systems, FieldScreen is a useful tool to follow species spread and invasions.

In our grassland assembly experiment differences between the two treatments in the dry grassland that had different starting diversity levels (testing priority effects) were detectable using photos taken with the camera mounted on the FieldScreen trolley. It is clear that the

S7 plot had a faster canopy closure than the S2 plot. Additionally, species identity differed between the systems with the S7 plot having a higher cover of legumes than the S2 plot. With the photos of the FieldScreen one can clearly see that the priority effects of which species arrive first have an influence on the development of the dry grassland over time. By comparing the photos in Figure 2, showing only one time point in each year, and the detailed photos in Figure 3 and 4, taken at several times within each year, indicate that classical ecological assessments often only done once or twice a growing season (due to manpower limitations) may miss important dynamics within the vegetation cycle. Continuous ecological monitoring systems, such as the FieldScreen have the potential to reveal novel properties during assembly.

For the future we aim to relate hyperspectral measurements with functional traits such as biomass, canopy N or leaf area index. We expect that hyperspectral and fluorescence data will open new paths to characterize changes in the Habitat Garden as a whole. It has the potential to give more information on overall vegetation traits over time than conventional ecological measurements. Thus the FieldScreen can be expected to be valuable tool to provide high-quality ecological data, and to help vegetation assessment, without the need to invest so heavily in human resources to measure multifunctionality. Additionally, its capability for much higher observation frequencies than conventional techniques will facilitate our attempts to describe fast ecosystem dynamics that happen at time-scales of hours to days, such as shoot development, leaf angle and flower opening. Further, as the FieldScreen can be dismantled and reconstructed rapidly, it can therefore be used repeatedly at more than one site for intensive intermittent monitoring, for example to measure stress via fluorescence or hyperspectral measurements over different vegetation patches.

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Manuscript 2

Sowing different mixtures in dry acidic grassland produced priority effects of varying strength.

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Original article

Sowing different mixtures in dry acidic grassland produced priority effects of varying strength

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ABSTRACT

Several longer-term assembly studies on ex-arable land have found that species that arrive first at a disturbed site can play a key role in the further development of the community and that this priority effect influences aboveground productivity, species diversity and stability of the grassland communities that develop. Restoration of nutrient poor, species rich grasslands is often limited by seed dispersal as well as the accessibility of suitable microsites for establishment. Sowing species (i.e. creating priority effects for further assembly) may help overcome such dispersal barriers, but the potential of using priority effects for restoration has not been tested in this type of dry grassland. We tested the hypothesis that sowing two different seed mixtures used for dry acidic grassland restoration onto a sandy substrate (which formed an equivalent to a primary succession) would create priority effects, and that these priority effects would be sustained over a number of years. We followed community assembly and measured aboveground productivity for four years after sowing. We found that priority effects caused by sowing of differently diverse mixtures did also occur in dry acidic grassland habitat, but that how persistent they were over time depended on the response variable considered. Priority effects on species number were not as strong as found in previous ex-arable land studies, whereas priority effects for aboveground productivity were still visible after 4 years. In addition, functional composition of the community still reflected the composition of the seed mixtures 4 years later. Our results suggest that priority effects can occur in nutrient-poor dry acidic grassland but in contrast to more nutrient-rich sites the breadth of responses affected may not be as wide.

1. Introduction

Semi-natural grasslands are hot-spots of European biodiversity with up to 50 species per m^2 (Peet et al., 1983). However, species-rich grasslands are currently threatened by both intensification and land abandonment, which has led to a drastic decrease in area over the last few decades (Kirmer et al., 2012). As such there is an increasing interest in restoring species-rich grasslands, which is often limited by dispersal-limitation (lack of target species propagules), microsite limitation in more nutrient-poor sites and excess nutrient loading of soils (Bakker and Berendse, 1999).

Much is now known about the often positive effects of plant diversity on ecosystem functions from biodiversity-ecosystem functioning experiments (Balvanera et al. 2006), where the diversity gradient is maintained via weeding. In more natural communities, factors other than diversity (such as land management, fertility of soils, climatic conditions or invasive species) are often considered more important key drivers of ecosystem properties (Milchunas and Lauenroth, 1993). However, there are very few studies that have addressed how important diversity effects are in relation to other ecosystem drivers (Flombaum and Sala, 2008; Tylianakis et al. 2008).

There is now increasing evidence that (at least for grasslands) sowing more diverse seed mixtures can create strong priority effects that are detectable in the vegetation long after sowing and that can drive a system as much as land-use history.

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Bezemer and van der Putten (2007) performed an experiment sowing either zero, four or fifteen species of plants onto ex-arable land then followed the dynamics of the system in terms of species turnover, productivity, temporal stability and diversity. In a grassland restoration context, Bullock et al. (2001, 2007) sowed either low or high diversity mixtures using plant mixtures typical for UK grassland restoration on a whole series of ex-arable sites and over a long period of time. Both studies found long-lasting effects (hereafter called priority effects) of initial sowing of seeds on further assembly, in particular when more diverse seed mixtures were sown. Flombaum and Sala (2008) removed species to create a plant species diversity gradient in the Patagonian steppe and found that aboveground net primary production increased with the number of plant species.

The restoration of species-rich communities is becoming a major tool to counteract biodiversity loss but it can also have positive effects on some ecosystem functions, for example increasing biomass production and hence nutrient cycling and reducing erosion (UNEP Nagoya Protocol, 2011). In a world experiencing increasing global change, where historical reference systems often no longer exist, ecological restoration is adapting by focusing as much on ecosystem functioning as on species composition when setting restoration goals (Choi et al., 2008. Hobbs et al., 2009).

1.1. Using priority effects for restoration

Priority effects occur when species that arrive first in an ecosystem significantly affect the further development of the community and thereby strongly influence community composition (Facelli and Facelli, 1993; Fukami et al., 2005). Priority effects can lead to lasting differences in species or functional group dominance, and hence can potentially drive ecosystem properties and functioning. Priority effects can occur on timescales from days to years and can be linked to differences in the arrival time of a species at a site but also to their success in establishing themselves in the community. Priority effects can short-term or lead to alternative stable states in vegetation (Grman and Suding, 2010). As such, restoration projects are often contingent upon priority effects in that they can alter vegetation trajectories as well as impede restoration success in some cases. The mechanisms underlying the priority effects or the timescale upon which they operate are rarely addressed however (Grman and Suding, 2010).

Most of the evidence for priority effects comes from grassland systems with intermediate soil nutrient availability (see Bezemer and van der Putten, 2007; Bullock et al., 2001, 2007). We do not know whether priority effects of sowing different mixtures can play a role in more nutrient-poor dry grasslands. Foster and Dickson (2004) hypothesise that systems with higher resource availability have more available niches but these niches are usually easily filled (packed) with species and this creates more neighborhood competition. In contrast in systems with more limited availability of resources such as soil nutrients, species attempting to establish themselves generally may find more open niche space but mainly face microsite limitation posed by abiotic conditions. Given this, one might expect that sowing differently diverse dry acidic grassland mixtures on sandy substrates (which form an equivalent to a primary succession) would overcome dispersal barriers but that microsite and nutrient limitations may deter establishment of target species more than on a more mesic substrate. The timescale upon which priority effects may operate in dry acidic grasslands may therefore be shorter than for more mesic sites.

Our field study aimed to test the strength of priority effects (in terms of detection of priority effects over time) in a dry acidic grassland created by sowing two different seed mixtures at time zero onto sandy substrate which formed an equivalent to a primary succession and asked the following two questions:

1) Does sowing two different seed mixtures produce priority effects in dry grassland, and how sustainable are they over time?
2) If there are priority effects which traits, processes or characteristics of the ecosystem do they relate to most?

In order to test this, we followed changes in different traits of the community over 4 years: at community level total species number (SN), community cover and aboveground peak biomass as a surrogate of productivity. We also assessed differences between responses of target (desired sown) and non-target species (invaders). At functional group level total species number, community cover and total aboveground productivity were split into functional groups (grasses, legumes, non-legume forbs).

2. Materials and methods

2.1. Experimental design

In autumn 2007 we established a grassland assembly experiment, the Habitat Garden, with two different grassland habitats (dry acidic and mesic grassland). The experimental field plots are on the campus of the Forschungszentrum Jülich, located in Jülich, West Germany (6° 22′0″E, 50° 56′0″N), and consist of 12 plots (randomized, each 2 × 2 m in size and separated by 50 cm paths sown with a non-clonal grass species). The dry acidic grassland plots ($n = 6$) were sown with two different diversity mixtures (whereas the mesic plots were sown with the same diversity of species at the start for removal experiments (not considered here, see also Plückers et al. 2013)).

The dry acidic grassland part of the experiment was designed to follow possible priority effects of sowing differently diverse mixtures on community assembly over time on a sandy substrate which thus formed an equivalent to a primary succession. For each dry acidic grassland plot the original soil was removed by digging out the soil to a depth of 40 cm, and a geomembrane permeable to water and nutrient laid down to avoid root input from plants growing outside the plots and to remove any seed bank. The sandy substrate, which consisted of sand (grain size 0.7–1.4 mm) mixed with one tenth potting soil (with very low nutrient and availability) was then filled into the prepared holes.

We sowed two differently diverse seed mixtures at a density of 3 g/m^2 using typical restoration mixes used to establish dry acidic grasslands in Germany (Rieger Hofmann GmbH Blaufelden, Germany), in December 2007. There were two diversity treatments ($n = 3$ per sowing treatment): S2 consisted of 2 grass and 25 forbs (one of which was a legume), and S7 consisted of 7 grass and 32 forb species (four of which were legumes). Species within the lower diversity S2 treatment formed a subset within the mixtures sown for treatment S7: e.g. the 2 grass species in S2 were also part of the S7 mixtures, the one legume species sown in S2 was also a subset of S7, Both mixture treatments had 12 non-legume forb species in common, whereas S7 has 16 additional different non-legume forb species and S2 has 12 additional different non-legume forb species. These seed mixtures were chosen, to ensure the study had some potential for regional restoration application in the future, such that we chose seed mixtures typically used by restoration practitioners and land managers in central Germany, provided by the wild seed company Rieger Hofmann GmbH. One quarter of each plot was not sown and kept as a control non-sown subplot. The experiment was fenced off to reduce confounding factors such as grazing by deer or wild boar. The plots were mown once a year in

August according to typical mowing regimes for such grasslands in Central Europe and were not fertilized.

Annual precipitation at the site over the 4 years (between 2007 and 2011) was 878 mm, 725 mm, 728 mm, 778 mm, 691 mm respectively (mean 700 mm over 50 years); mean annual temperatures in the same time span were 11,1 °C, 10,5 °C, 10,5 °C, 9,2 °C, 11,2 °C (mean 9.9 °C over 50 years) (measured at the local weather station in the Forschungszentrum Jülich). There were no notable weather extremes (e.g. 100 year climate extremes) during this time, except that mean annual temperature in 2011 was somewhat higher than the long-term mean.

2.2. Plant census and sampling, soil sampling

In the first year (2008) we did not mow to allow initial establishment without disturbance, whereas thereafter hay was cut in late July/early August at peak vegetation. Every year before mowing, a vegetation census assessed cover of every species using a decimal scale based on Braun Blanquet but modified by Londo (1976). Total community cover can therefore sum to more than 100% since the canopy is complex and multilayered. Total aboveground biomass production (dry matter yield, g/m^2) was measured in two 0.1 m^2 quadrats (20 × 50 cm in size) per plot (one randomly positioned at each harvest in the remaining plot and one in the control area). All aboveground plant material per quadrat was cut 2 cm above the soil surface and samples were dried at 70 °C followed by measurement of total community aboveground dry weight. In 2010, 2011 biomass was sorted into three functional groups: legumes, non-legume forbs and grass species. We based these categories on previous functional diversity studies we have performed where these functional groups were shown to perform varying functions within grassland habitats; (e.g. Temperton et al. 2007).

Pooled soil samples were taken per plot once a year in spring or summer (the first two years in spring (March), the last two years in summer (August)) from the topsoil layer (0–15 cm) and analyzed for total C and N content. At time zero (December 2007) one pooled soil sample of soil substrate was analyzed for soil chemistry. For total soil N and C (% weight), the soil samples were dried (12–24 h, 70 °C), ground to a homogenously fine powder and 2–100 mg sample was burned in an elemental analyzer (System: VarioelCube or Leco).

2.3. Statistical analysis

Our experiment is a one factorial experiment testing effects of the factor sowing diversity with two levels of medium and high diversity. Response variables measured were total species number, total cover and total aboveground biomass production per growing season measured at peak biomass in August of each year. However species number, cover and total aboveground biomass production per functional group were also measured, as well as species number and cover of target (sown) and non-target species.

We tested priority effects over 4 years, such that we analyzed most of our data (see Table 1) using Repeated Measure analysis of variance (RMANOVA). Repeated Measure ANOVA was done using univariate procedures with a Sphericity correction to allow for differences in time intervals between measurement dates. All data that did not conform to homogeneity of variance or normal distribution were transformed before analysis: count data (i.e. species number) were square root transformed and all other data multiplied to the power of a specific factor. This specific factor was derived by a power-transformation test to achieve normality of the residuals and homogeneity of variance. This is a useful preprocessing technique and robust against outliers. Back-transformed means and standard errors from the analyses are presented throughout in graphs and tables. All analyses were computed using the Program R. To analyze any treatment difference within single years we used the two-sided student t-test ($p < 0.05$).

Table 1
Results of Repeated Measures ANOVA testing the effect of sowing treatments, time (Year) and their interaction on response variables. Sowing treatment effects show results of testing over the whole time span, whereas Year effects describe how the effect of the sowing treatment factor changed over time. Note this is a summary table of many analyses, such that each line represents a single RMANOVA analysis.

Response variable	Factors					
	Sowing treatment		Year		Year X sowing treatment	
	d.f.	P	d.f.	P	d.f.	P
Total species number	1	0.346^{nsd}	3	0.003**	3	0.2^{nsd}
Forb species number	1	0.238^{nsd}	3	<0.001***	3	0.936^{nsd}
Legume species number	1	0.018*	3	0.004**	3	0.088^{nsd}
Grass species number	1	0.156^{nsd}	3	0.18^{nsd}	3	0.05*
Target species number	1	0.097^{nsd}	3	0.02*	3	0.18^{nsd}
Non-target species number	1	0.29^{nsd}	3	0.002**	3	0.27^{nsd}
Ratio target to non-target species number	1	0.23^{nsd}	3	0.006**	3	0.22^{nsd}
Total cover	1	0.828^{nsd}	3	<0.001***	3	0.11^{nsd}
Forb cover	1	0.02*	3	<0.001***	3	0.081^{nsd}
Legume cover	1	0.005**	3	0.01**	3	0.67^{nsd}
Grass cover	1	0.777^{nsd}	3	0.001***	3	0.28^{nsd}
Target species cover	1	0.019*	3	<0.001***	3	0.032*
Non-target species cover	1	0.186^{nsd}	3	0.004**	3	0.202^{nsd}
Ratio target to non-target species cover	1	0.058	3	0.32^{nsd}	3	0.11^{nsd}
Total biomass	1	0.022*	2	0.024*	2	0.05*
Forb biomass	1	0.0048**	1	0.56^{nsd}	1	0.40^{nsd}
Legume biomass	1	0.007**	1	0.53^{nsd}	1	0.68^{nsd}
Grass biomass	1	0.16^{nsd}	1	0.19^{nsd}	1	0.81^{nsd}
Soil %N	1	0.072	3	<0.001***	3	0.243^{nsd}
Soil %C	1	0.043*	3	0.005**	3	0.51^{nsd}

$^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$; nsd no significant difference.

3. Results

3.1. Is there a priority effect through sowing differently diverse mixtures on diversity and productivity?

Between 2008 and 2011 total species number and total cover of the communities increased over time for both sowing treatments (Fig. 1A and C, Table 1 significant year effect). Sowing different starting diversities affected the total aboveground biomass production and functional group composition and productivity diversity significantly over the whole 4-year time span (Fig. 1B and D and Fig. 2 respectively, Table 1). There was a trend towards total species number and community cover being affected by sowing but only in the first year (t-test $p = 0.056$, Table 1 over 4 years no significant treatment effect).

The number of legume and forb species increased over time in both sowing treatments (Fig. 1B, Table 1 significant year effect). Forb species number was unaffected by sowing treatment whereas there were more legume species in S7 plots in all years (Fig. 1B, Table 1 significant treatment effect). Changes in grass species number were affected by the sowing treatment, with the proportion of grass species over total species increasing in S2 over time (Fig. 1B, Table 1 significant interaction effect)

Cover of forbs, legumes and grasses increased over time (Fig. 1D, Table 1 significant year effect). In all years legumes had a higher cover in the S7 than S2 plots, but a lower cover of non-fixing forb species (Fig. 1D, Table 1 significant treatment effect).

Total aboveground peak biomass (as a surrogate of productivity) increased over time for both sowing treatments (Fig. 2A, Table 1

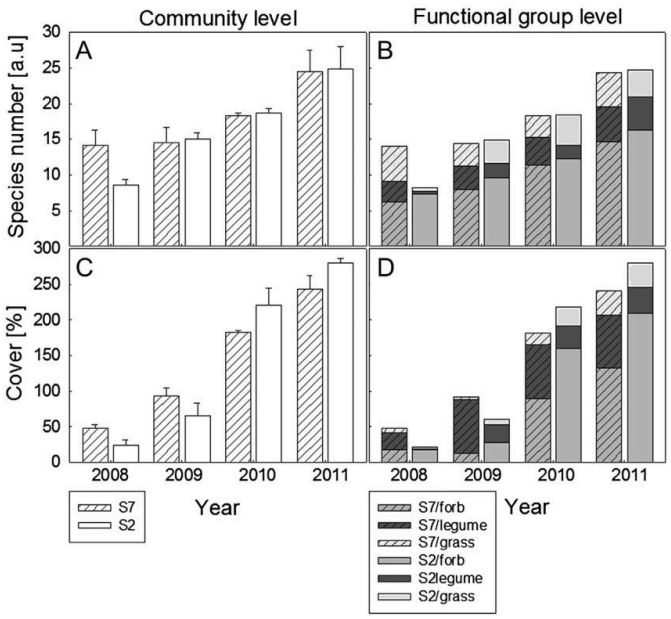

Fig. 1. The development of species number and cover at the community and functional group level. Values are means (+one standard error of the mean) **A and C**: Community level: note that plant cover for a plot can be higher than 100%, because of 3-D aspects of plant community canopies. **B. and D**. Functional group level: non-legume forb species (bottom section), legume species (middle section) and grasses (top section).

significant year effect), but the detailed development of both treatments was different over time, which was confirmed by a significant interaction effect between treatment and year effects (Fig. 2A, Table 1).

The S7 plots had a higher total aboveground biomass production in all years (Fig. 2A, Table 1 significant treatment effect). Total aboveground biomass of legumes was much higher in the S7 plots than in the S2 plots, whereas forbs dominated more in S2 plots (Fig. 2B, Table 1 significant treatment effect). The relative aboveground biomass production of grasses, forbs and legumes did not differ significantly between 2010 and 2011 (when measured). Total aboveground biomass production increased in 2011 in the S7 plots, due to a relative increase in legume biomass (especially of *Lotus corniculatus*). The high variability of the error bars for 2011 biomass can be mainly attributed to the presence of one woody legume shrub in one plot (*Genista tinctoria*).

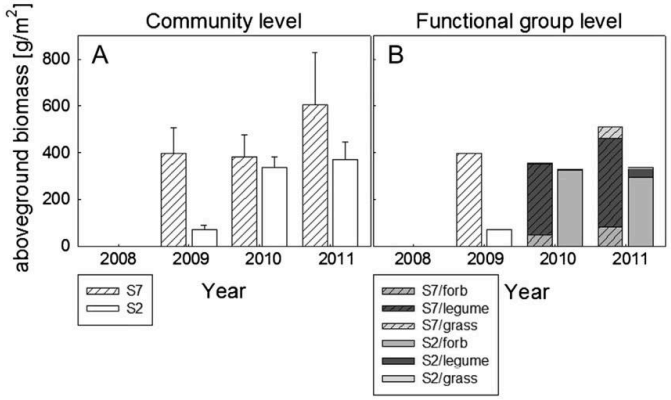

Fig. 2. The development of aboveground biomass at the community and functional group level. Values are means (+one standard error of the mean) **A**. Community level **B**. Functional group level (as in Fig. 3). The total aboveground biomass values of the community level and the sum of the functional group level is not the same because of transformation procedures.

3.2. Is there a priority effect through sowing differently diverse mixtures on establishment of target species and functional groups over time?

Invasion of species increased over time but species richness was not significantly different between treatments (Table 1 see Fig. 3A total number of species). Species number and cover of non-target species (non-sown species) and target species (sown species) of both sowing treatments increased over time (Fig. 3A, Table 1 significant year effect). There was a significant effect of the sowing treatments on the cover of target species (Table 1 significant sowing treatment), but no significant effect of the sowing treatments on the number of non-target species and their cover (see Table 1 no treatment effect overall years). The ratio of target to non-target species number significantly decreased over time as newcomers arrived (Table 1 significant year effect, Table 2 for changes in percentage target species). At the beginning 27 species were sown in the S2 plots and 39 species in the S7 plots. Only 30–40% of the sown species had established after 4 years in 2011 (Table 2), with both sowing treatments having nearly 30% target species in the first year (2008) but had slightly higher establishment in the S2 plots than S7 by 2011 (Table 2). Overall, S7 plots had a higher establishment of target species (relative to non-target species) than S2 plots (Table 2), but the proportion of established target species in the S2 plots significantly increased from 2008 to 2011 (*t*-test $p = 0.050$, data not shown), whereas this was not the case in S7. The non-target species that managed to establish did not mainly derive from the surrounding mesic grassland plots, but were ruderal species such as thistle (*Cirsium*, *Equisetum*, *Rumex* or *Polygonum* sp). Non-target dry acidic grassland species from other sandy plots formed an intermediate group of species that successfully managed to invade at a later time point after sowing. This is as one might expect, given the number of sown species that the treatments shared in common, such that quite a few of the sown dry acidic grassland species in S2 treatments were a subset of the species in S7. In detail, there were 12 "new" non-sown target species that could potentially invade from S2 to S7, versus 24 options from S7 to S2. Our results follow the expected relative success of target species (those sown on a treatment) with high invasion from S7 to S2 than the other way. Over the 4 years, the sowing treatment had a significant effect on assembly. The control plots had significantly less total species number, total cover and total aboveground biomass production over the whole time than the sown plots (data not shown).

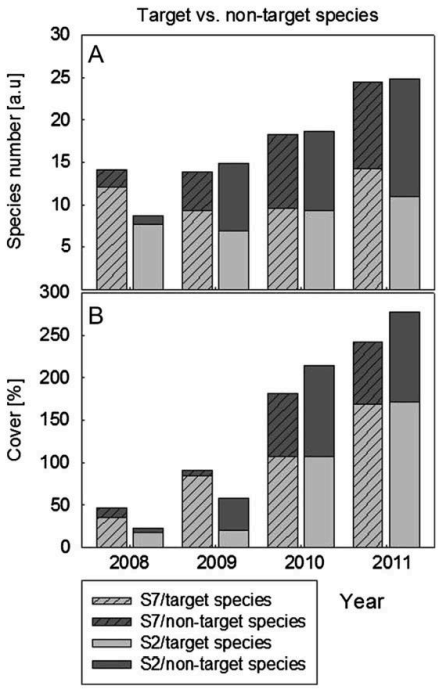

Fig. 3. Sowing treatment effects on the development of mean species number (A) and cover (B) separated into target species (sown species, bottom section) and non-target species (non-sown species, top section).

Table 2
Establishment of target species in the first and fourth growing seasons (2008 and 2011) in relation to the total number of species sown in the original mixtures in 2007. Data show percent values (%) ± standard error.

Sowing treatment	% Establishment of target species	
	2008	2011
S2	28.39 ± 2.5	40.74 ± 3.7
S7	31.62 ± 5.2	36.75 ± 4.3

3.3. Did sowing differently diverse mixtures affect soil conditions?

Sowing different starting diversities significantly positively affected total soil C (% C), with higher soil C in the S7 plots over the whole time span (Table 1 significant treatment effect). Total soil C generally increased, but between 2010 and 2011 it decreased across all plots.

There was a positive trend that sowing different diversities affected total soil N (% N) (see Table 1), with higher soil N in the S7 plots. Total soil N was higher by 2011 compared to the time zero soil samples without species (time zero: 0.005% weight, 2011: S7 plots 0.02% weight and S2 0.01% weight). Total soil N increased until 2010 and then decreased (see Table 1 significant year effect for soil % N).

4. Discussion

4.1. Does sowing differently diverse mixtures produce priority effects in dry grassland, and how sustainable are they over time?

Sowing two different mixtures typically used for restoration of dry acidic grassland in central Europe caused priority effects that influenced community assembly 4 years after sowing. Not all response variables measured, however, still showed evidence of a priority effect after 4 years. The longest lasting priority effects related to aboveground peak biomass, community cover and functional composition of the original seed mixture (whereby the mixture sown was still significantly affecting functional group composition in the extant community after 4 years).

Our priority effects were however not as wide in their effects as those found in experiments on more nutrient-rich ex-arable land (Bullock et al. 2001, 2007; Bezemer and van der Putten, 2007) where initial sowing significantly affected total species number as well as aboveground biomass and stability of the system. Bezemer and van der Putten (2007) could still see differences due to sowing zero, four or fifteen species on ex-arable land after 9 years. In general, these studies and our study indicate that one needs to follow a range of response variables, since the length of time over which a priority effect influences assembly or functioning is clearly

dependent on which variable one measures. It would be interesting to follow more ecosystem functions than just aboveground productivity in further studies, as well as typical vegetation measures. One possible reason for the milder priority effects we found on dry acidic grassland compared to mesic conditions, could be that in communities developing on very sandy substrates, the key limitations to establishment (apart from dispersal) are abiotic in nature (e.g. microsite limitation) and less driven by direct interactions between plant species already present (see Fig. 4). As Foster and Dickson (2004) highlight in their conceptual model of how invasion is modulated by available nutrient resources for plants: systems with higher resource availability experience more niche packing and hence more competition than systems with lower resource availability. Invading species experience lower microsite limitation however, since abiotic conditions are more benign. Sowing more diverse seed mixtures should initially increase the niche packing speed since it removes dispersal limitation, however at later successional stages the area with less seeds sown should allow more new invaders to establish than the high diversity site. In contrast in more nutrient-limited systems, species attempting to establish should generally find more open niche space but mainly face microsite limitations posed by abiotic conditions, as we found in this study. Sowing more diverse mixtures should initially decrease dispersal limitation and increase establishment of target species. In later succession (in contrast to the higher resource scenario) a higher proportion of the established species will be new invaders (non-target) since a lower proportion of the sown species will be able to establish due to microsite limitation. During later succession, facilitation by nurse plants may help new invaders to establish, and the potential for this may increase in more diverse sites.

By sowing two differently diverse seed mixtures on a sandy substrate we influenced the availability of propagules and reduced the dispersal limitation typical of such nutrient-poor grassland communities in this early stage of assembly. We made species available through sowing and gave these species the chance to establish themselves first and thus cause priority effects in further assembly. Considered within filter theory (Kelt et al., 1995): to establish themselves, our sown seeds (target species) had to pass through the mesh of the abiotic filter (since the sandy substrate formed an equivalent to a primary succession with extremely low nutrient and water holding capacity). The sown target species did not however have to overcome a biotic filter resulting from plant species already present in the habitat at the moment of their arrival. At this time point we expect that microsite limitation will have affected the germination and establishment success of the seeds the most. Our establishment data (Tables 2 and 3) back this up, with around only 30% of the sown target species managing to establish in the first growing season, and around 40% managing to establish by 2011 in both sowing treatments. Our data fit in well with results from central European grassland restoration projects (Kiehl et al. 2010) where 4 years after sowing (also at a density of 3 g/m^2) around 30–80% of target species had managed to establish, and the lower establishment rates were mainly on low-nutrient sites. This suggest that in our study microsite limitation was playing a role and affected species richness of the vegetation, even if we did not directly measure it. Kiehl et al. (2010) assessed various techniques for introducing species to a site during restoration in Central Europe and found that although sowing could overcome dispersal limitation, the long-term success of restoration also depended very much on the availability of appropriate abiotic conditions including establishment microsites.

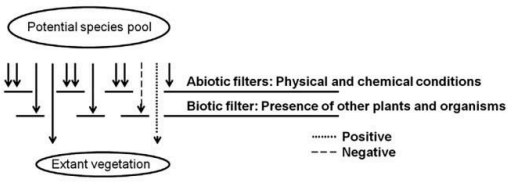

Fig. 4. Conceptual model of filter theory of community assembly based on Kelt et al., 1995 (modified from Hobbs and Norton, 2004) and adapted to include facilitation as well as competition as part of the mechanisms behind the biotic filter. The dotted arrow highlights when facilitation can help a species manage to establish, e.g. if a nurse plant provides a microsite or additional nitrogen during germination and early growth. The strongly dotted arrow indicates negative competitive interactions potentially blocking a species from managing to establish.

In classical biodiversity-ecosystem functioning experiments (where natural assembly is not allowed) more diverse communities are generally more resistant to invasion (Roscher et al, 2009). Huang et al. (2013) found in a prairie biodiversity experiment where weeding ceased after 3 years, that the positive relationship between diversity and productivity persisted even after cessation of weeding. In our study invasion pressure did increase total species number but the lack of significant difference in species number between the sowing treatments (except in year 1) suggests that invasion resistance may have been similar between treatments (Fig. 1). Overall, S7 plots had a higher establishment of target species (relative to non-target species) as well as higher soil C content and a trend to higher N content than S2 plots (Table 1). The proportion of established target species in the S2 plots significantly increased from 2008 to 2011, whereas this was not the case in S7, despite soil C remaining higher in the S7 treatment. So S7 started out with higher establishment success for target species but S2 caught up over time (Table 2). This is mirrored in the cover data where S2 started out having lower community cover than S7 but become significantly higher than S7 by 2010 and 2011 (see Fig. 1C, see 3B for target/non target species cover). This shows that the two treatments may have had similar invasion of species (in terms of numbers) but that the abundance of the species differed.

It seems that time plays a crucial role in restoration success for establishing desired target species in species-rich grasslands. Baasch et al. (2012) evaluated restoration experiments in ex-mining sites on sandy soils in eastern Germany and found that the species-rich grasslands established after hay transfer or sowing were highly resistant to invasion of ruderal species (despite hay transfer methods not only having positive effects on establishment). After 9 years, however, there was no difference between treatments in terms of total vegetation cover, species richness and the number of target species. In our study we found the same effect after only 4 years: there was no difference between sowing treatments in terms of total species number and number of target species, although productivity, community cover and functional composition did vary.

Focusing on the different functional groups of the species sown (e.g. legumes, non-legume forbs and grasses) our study found that the functional group composition sown was still detectible in the vegetation 4 years after sowing. This is an interesting effect, even if we cannot separate effects of the species richness from the functional richness of the seed mixtures.

This detectable priority effect after 4 years was valid for cover and biomass of forbs and legumes, and richness of legumes only (Table 1) but not for grasses. While one can see in our data that the higher proportion of legumes sown was reflected in higher cover and biomass of legumes over time, this effect was not found for the forbs. For the forbs, the S2 treatment that started out with less forb species, had an as high proportion of forbs in the community after 4 years as the S7 treatment. The particularly strong priority effect of sowing legumes on legume composition may be related to the finding that legumes established quickly and well. They seemed to

be well adapted to the conditions on site from the start and established well in S7, whereas in S2 the one legume species did not establish well, allowing the non-legume forbs to become dominant. We know from many greenhouse and field studies with legumes interacting with other functional groups, that legumes tend to be competitive across a range of abiotic conditions (Temperton et al., 2007; non-published data).

If in follow-up experiments the functional composition of the mixture were found to be more important than the species richness, one could perhaps use priority effects of initial sowing composition to direct the functional composition of the community as well as total aboveground biomass and cover. This is turn could potentially have positive effects on nutrient cycling and carbon sequestration in mesic grasslands (*sensu* Steinbeiss et al. 2008; Oelmann et al. 2011). This would need to be tested in separate experiments before being applicable to restoration since seed mixtures are rarely separated into these groups, but just sown as higher or lower diversity.

5. Conclusions

Overall, our study aimed to test whether priority effects of sowing differently diverse seed mixtures play a role in dry acidic grasslands, and we found that they do, but how sustained they were depended on the response variable measured. Our study found relatively low establishment success of target species, but the results are in line with results from low-nutrient grassland restoration, suggesting that microsite limitation and related filtering effects of severe abiotic environments may be the strongest driving factors in assembly of dry acidic grassland. Additional reduction of microsite limitation via planting out nurse plants to facilitate establishment in such harsh conditions or including an intermediately severe disturbance regime (as in Jentsch et al. 2009) may be as important to improving dry grassland restoration success as sowing therefore.

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Manuscript 3

Priority Effects of Time of Arrival of Plant Functional Groups Override Sowing Interval or Density Effects: A Grassland Experiment.

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Priority Effects of Time of Arrival of Plant Functional Groups Override Sowing Interval or Density Effects: A Grassland Experiment

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Abstract

Priority effects occur when species that arrive first in a habitat significantly affect the establishment, growth, or reproduction of species arriving later and thus affect functioning of communities. However, we know little about how the timing of arrival of functionally different species may alter structure and function during assembly. Even less is known about how plant density might interact with initial assembly. In a greenhouse experiment legumes, grasses or forbs were sown a number of weeks before the other two plant functional types were sown (PFT) in combination with a sowing density treatment. Legumes, grasses or non-legume forbs were sown first at three different density levels followed by sowing of the remaining PFTs after three or six-weeks. We found that the order of arrival of different plant functional types had a much stronger influence on aboveground productivity than sowing density or interval between the sowing events. The sowing of legumes before the other PFTs produced the highest aboveground biomass. The larger sowing interval led to higher asymmetric competition, with highest dominance of the PFT sown first. It seems that legumes were better able to get a head-start and be productive before the later groups arrived, but that their traits allowed for better subsequent establishment of non-legume PFTs. Our study indicates that the manipulation of the order of arrival can create priority effects which favour functional groups of plants differently and thus induce different assembly routes and affect community composition and functioning.

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Introduction

Research into the assembly of ecological communities has shown that the extant composition of communities is strongly influenced by historical factors [1–3]. Priority effects occur in communities, when one (or more) species already is present in a habitat and thereby affects the success of later species [4,5], and this effect can be either negative, positive or neutral [6]. The success of other species can relate to their establishment, growth or reproduction [7]. Priority effects are thus important e.g. to understand when applying ecological theory and knowledge to help restore degraded habitats where certain species are introduced to a site via restoration (Grman and Suding 2010). Species arriving prior to other species are generally considered to either affect newcomers via size-asymmetric competition [8] or so-called legacies in the soil created by effects of plant-soil feedback on the soil [9]. Another possible mechanism of priority effects is nitrogen (N) facilitation (including N transfer and N sparing) between N_2-fixing species arriving early during assembly and other functionally different species arriving at a later time-point (see Körner et al. [10] for first indication of this). No matter the mechanism, the outcome of priority effects seems to be that competitive and or facilitative interactions for newcomers are altered.

Priority effects can lead to lasting differences in species or functional composition, and hence can potentially drive ecosystem properties and functioning, and may sometimes even have a stronger influence than the effects of abiotic conditions on community composition [1,11]. In aquatic model-ecosystems also, there is evidence that properties, such as biomass production or community size, seem to be more dependent on initial arrival order and frequency than on other factors such as initial species richness [4,12]. Recent research has found a mediating role of soil resource availability in relation to the importance of priority effects, however, at least in a pot experiment [13].

Recent research has focused on two different kinds of priority effects in plant assembly, the one showing long-term effects on vegetation caused by adding species mixtures at the same time [14,15] or altering the sequence of arrival of different species or groups of species [10,11,13]. Although the simultaneous introduction of species is of high relevance to restoration projects where mixtures of plants are often used, the potential mechanisms of order of arrival of in particular different functional groups has not been much explored yet. N_2-fixing legumes are known to be

ecosystem engineers, in particular introducing extra N_2 into soils and hence driving N cycling and community productivity [16]. We now know from many biodiversity experiments that niche complementarity between species varying in traits can lead to better overall resource-use at community level, and that particular combinations of functional groups (particularly N_2-fixers combined with grasses) as well as species richness can drive positive diversity effects [17–20]. It may be that this complementarity between different functional groups is a driver of assembly over time, and hence composition and functioning of communities.

Körner et al. (2008) varied the arrival order of three different plant functional types (from hereon called PFTs) each containing two out of six plant species in microcosms, with either legumes, non-legume forbs, or grasses sown first and the other two groups sown three weeks later. They found strong priority effects of sowing legumes first on both aboveground and belowground community productivity, even after two growing seasons. In their study the set of species in each microcosm was comparably small in relation to the biodiversity of common grasslands in central Europe. To be able to set the outcome of such a study into a more applied context (e.g. restoration or creation of semi-natural grasslands) it is essential to look on the species which occur naturally in such environments. In particular to enhance restoration of species-rich grasslands, the role of legumes as possible ecosystem engineers on nutrient-poor soils needs further research.

As the number of species in a system increases so does the number of possible interactions, either positive or negative thus affecting assembly [21–23]. We know from many biodiversity experiments that niche complementarity between species varying in traits can lead to better overall resource-use at community level, and that particular combinations of functional groups (particularly N_2-fixers combined with grasses) as well as species- and functional group richness can drive positive diversity effects [17–20].

Species that arrive first at a site have a competitive advantage over those that arrive later, and the longer the time interval between establishment episodes the more asymmetric competition may become [8]. The relative benefit one PFT can get through this competitive advantage of arriving first, however, might become a benefit for the whole community when these species have special traits such as legumes due to their ability to increase N availability either via N sparing or via N transfer. Therefore especially in harsh environments (e.g. low initial nutrient content or high environmental stress) legumes may have a positive effect not only on productivity but also on other species survival and establishment and thus positively influencing assembly [24]. Positive effects found on productivity by sowing legumes before other functional groups [10] were related to a three-week sowing interval. To what extent the sowing interval affects assembly outcomes now needs further study, since the ontological state (life stage) of a plant population may influence the species interactions and hence priority effects.

Community assembly in general and priority effects are in all likelihood modulated by both density of individuals in communities as well as environmental resource availability [8]. The law of constant yield predicts that even-aged populations grown in different densities show the same overall productivity after a certain period of time [25]. Where initial biomass is higher with increasing density this relationship wears off with time leading to the same productivity of standing biomass independent of the population density (with higher individual numbers in high densities but lower standing biomass per individual). Competition for nutrients is considered the key mechanism behind the constant yield law, but size-density relationships may change in different environments [26]. Under more extreme environmental conditions, for example, facilitation may drive communities as much as competition does. The size of an individual does not necessarily decrease with increasing density. Indeed, if facilitation and competition take place simultaneously, the size of individuals may even increase with density. In addition, sowing at high densities is often associated with higher cover values and relative abundance of sown species [27,28] correlating with greater productivity. In this sense increasing sowing density could potentially have a positive effect on productivity. However size-density-yield relationships especially in mixed stands have rarely been investigated.

This study investigated the effect of order of arrival (priority effect) of functionally different species groups (PFTs) on the productivity as well as species and functional composition of species-rich grassland communities grown in pots under greenhouse conditions. The experiment was multi-factorial regarding order of arrival, density and sowing interval as factors tested for their effects on community productivity and composition. The following hypotheses were tested:

1) The longer the sowing interval between the PFT sown first and the subsequently sown PFTs the lower the aboveground productivity of the system will be. This is due to stronger asymmetric competition between PFTs when early arrivers get a head-start and very little complementarity between PFTs can occur.
2) Sowing different seed densities will result in higher individual numbers at higher sowing densities but overall aboveground productivity will remain the same across all levels of the density treatment due to the law of constant yield.

Materials and Methods

Experimental Setup and Initial Conditions

A pot experiment was set up in the greenhouse of the Institute of Bio- and Geosciences (IBG-2), Germany in April 2011 sowing seeds typical of mesic and dry grassland habitats in the region. The experiment lasted from May until August (a total of 18 weeks from first sowing to harvest). A total of 28 typical central European grassland species were selected belonging to the three plant functional types forbs, grasses and legumes (PFT: 14 forb-, 7 grass-, 7 legume species; for species list see Supporting Information, Table. S1: Plant species per functional group with respective seed mass per pot). We chose this relative contribution of the three PFTs based on relative abundances in natural or semi-natural communities in such grasslands in Germany, (Matthias Solle, personal communication) known to have different effects on nutrient cycling and productivity from biodiversity experiments [18,29]. Species selection was based on broad phytosociological units of the given grassland communities in dry to mesic conditions [30] and Ellenberg's indicator values [31].

Experimental Design

Pots with a volume of 5 litre and an upper diameter of 20 cm and a diameter of 15 cm at the bottom were filled with a 1:2 mixture of sand (grain size 0.7–1.4 mm) and low nutrient potting soil (Einheitserde- und Humuswerke GmbH & Co. KG; "Typ P") as a substrate (for initial nutrient status of the soil see Supporting Information, Table. S2: Results of soil analysis at the beginning of the experiment). By using a substrate with low initial nutrient status we wanted to foster effects of positive and negative plant-

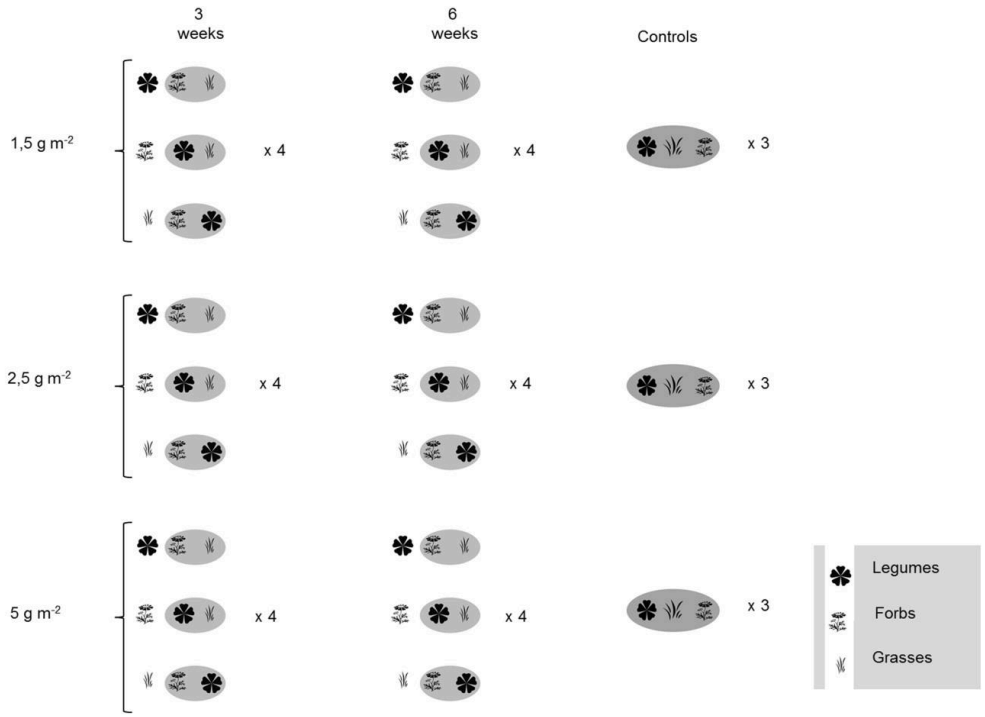

Figure 1. Experimental setup showing the three different treatments of the experiment: the priority effect treatment of arrival order, the different density treatment and the sowing interval treatment. Priority effects of early sowing of one PFT are shown with a plant functional group symbol without a circle, and later sowing of the remaining two PFTs are shown in grey circles. For the priority effect treatment legumes, forbs or grasses were sown a number of weeks before the other two groups. Density levels were 1.5, 2.5 and 5 g m^{-2}, and sowing intervals were three- and six-weeks between first PFT sown and remaining PFTs. Controls involved all PFTs being sown together at the same time. Number of replicates is shown in bold next to each treatment.
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plant interaction to be reflected in productivity and species composition. Sand was added to increase water permeability.

Plant species density was standardised on behalf of their seed mass (giving a sowing density) and records of germination capacity. Seeds were obtained from Rieger-Hofmann GmbH and mixed manually to form a density treatment with three different levels (1.5; 2.5 & 5 g m^{-2}). Before sowing, densities were calculated on the basis of the thousand-seed weight (*TSW*) of each of the species (for species list see Supporting Information, Table. S1: Plant species per functional group with respective seed mass per pot) and an empirical value derived from germination tests (*A*) standing for number of individuals of species "x" m^{-2} (Matthias Stolle, Rieger-Hofmann GmbH, personal communication) for pot surface area (*B*) and a factor (*Y*) to meet the desired plant density level, as follows:

$$((A * TSW(g))/1000) * Y) * B$$

Assembly order was influenced through a variation in order of arrival (sowing time) of three different PFTs. Species groups referred to as PFTs were non-legume forbs (hereafter referred to as forbs), nitrogen-fixing Fabaceae (legumes) and grasses. Four priority effect treatments (PE) were set up: forbs sown first (F-first), grasses sown first (G-first), legumes sown first (L-first) and a control treatment with all PFTs sown together at the same time. The priority effect treatment was created by sowing one PFT first on one sowing date (13-April-2011) and the other two remaining PFTs respectively three-(04-May-2011) or six weeks (25-May-2011) later providing a sowing interval treatment of either three- or six weeks. Each priority effect- and sowing interval treatment was additionally sown at three different density levels giving four replicates per PE-, density- and sowing interval-treatment (Fig. 1).

Pots were watered continuously by an automated irrigation system (Gardena) using rain water. Water was allowed to drain from the pots through holes in the bottom. Temperatures in the greenhouse varied from 17°C at night to 25°C in the day during the experimental period. Sowing occurred in all 81 pots one week after the filling of to allow the substrate to rest. Three soil samples were taken at time zero to evaluate the nutrient status at the beginning of the experiment (Supporting Information, Table. S2: Results of soil analysis at the beginning of the experiment). Pot

Table 1. ANOVA table for the effects of experimental treatments on aboveground biomass.

Treatment	S.S.	d.f.	M.S.	F	p	Partial-η^2
PE	1.667	3	.556	82.527	.000	.813
Sowing_Interval	.399	1	.399	59.313	.000	.510
Density	.075	2	.037	5.567	.006	.163
PE * Sowing_Interval	.151	3	.050	7.466	.000	.282
PE * Density	.040	6	.007	.983	.445	.094
Sowing_Interval * Density	.004	2	.002	.307	.737	.011
PE * Sowing_ Interval * Density	.038	6	.006	.937	.476	.090
Error	.384	57	.007			

ANOVA table for effects of the experimental treatments (arrival order (PE), sowing density (density) and sowing interval) and their interactions on aboveground biomass production. Effect sizes are calculated as partial η^2.
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distribution followed a randomised design and pot positions were changed at one time point during the experiment to take account of microclimate effects. In the case of colonisation by non-target species, pots were weeded (four times during experiment).

The response variables measured were: aboveground biomass, cover and number of individuals per plant species.

To identify treatment effects on plant community composition we assessed plant cover per species at one time point during the experiment at the time point of peak biomass development, 81 days after the first initial sowing. These estimates were performed using a modified cover estimation method following Braun Blanquet and further modified by Londo [32]. In addition to estimated cover per plant species, numbers of individuals per species were counted in each pot.

At the end of the experiment, total aboveground biomass was determined through a destructive harvest (for each of the two sowing intervals it was 78 days after the second sowing). Although the start of both sowing interval groups was at the same time, the end of the experiment was at two different time points depending on the sowing interval treatment (21-Jul-2011 & 12-Aug-2011). The different harvesting dates for these two groups (three- or six-week interval) were chosen to allow the latter sown remaining PFTs to have the same time to develop in both sowing interval treatment groups. At harvest aboveground plant parts were cut 2 mm above the soil surface, separated into PFTs, and oven-dried at 70°C to constant weight. For the first harvesting date (21-Jul-2011) only one of the three control replicates was harvested, leaving the remaining two for the second harvesting date. In addition, soil samples were taken from each pot to evaluate the nutrient status for nitrate, nitrite, ammonia, phosphorus and potassium. Measurements were performed after extraction with an 1 M KCl solution and following measurement in a Dionex ICS-3000 (except for potassium which was analysed in an 0.1 M CaCl solution with an ICP-OES). Total carbon and nitrogen in the soil were measured using an element analyser (VarioelCube, Elementar).

Statistical Analysis

The experiment was multi-factorial in design with three main factors: priority effect of arrival order, sowing interval and density. The priority effect factor had four different levels (F-first, G-first, L-first and control sown at the same time). The sowing interval factor had two levels (three- and six weeks between early sowing of first PFT and subsequent sowing of the other two PFTs). The density factor had three levels (1.5, 2.5 and 5 g m^{-2} seeds sown). Response variables included aboveground biomass at community level and at population level plant cover per species, to assess species composition but also community structure.

Number of plant individuals per pot were analysed using a one-way ANOVA testing for the effects of density and sowing interval independently. Treatment levels were tested against each other by performing Tukey contrasts. This method enabled us not only to test for general treatment effects but to test each single level of a treatment specifically in relation to each other without increasing the chance of a type one statistical error.

Communities' similarities were depicted by a dendrogram resulting from a hierarchical cluster analysis on the basis of a distance matrix (between group linkages). Distances were calculated on behalf of individual species' occurrence and cover by using Pearsons' correlation coefficient.

Biomass data was analysed using three-way ANOVA testing for effects of the factors PE, sowing interval and density as well as any interaction effects between these factors (for ANOVA Table see Table. 1). The experimental design was almost balanced and orthogonal for the three factors, except that for the three controls replicates (i.e. all PFTs sown at same time), one replicate was harvested at the first harvesting date and the remaining two at the second harvesting date. Data was generally analysed using Type III ANOVA but also using Type I ANOVA. Type I ANOVA allows to alter the order and thereby take into account the relative variability explained by this factor (see Oelmann et al. [33]) depending on when it is fitted in the model. Type I allowed us to therefore test relative effects of the three factors, depending on when they were fitted in the model.

Normal distribution of the residuals and homogeneity of variance were checked with pp-plots and Levene's tests respectively. Any data that did not fulfil the assumption of homogeneity of variance and normal distribution of the residuals were transformed (log 10) before analysis. Effect sizes for each factor as the proportion of explained variance were calculated as partial η^2. Analyses were run using PASW Statistics 18 (formally known as SPSS; IBM).

Results

Priority Effect of PFTs on Aboveground Productivity

The early sowing of one PFT (PE treatment) had a significant effect on aboveground plant productivity (Fig.2; $F_{(3,\ 57)} = 82.527$, $P < 0.0001$).

Within the levels of the priority effect treatment, communities in which legumes were sown first (L-first) were the most productive (especially when sown at high density) with aboveground biomass ranging from 664±92 g m^{-2} to 1608±126 g m^{-2} followed by G-first (ranging from 521±37 g m^{-2} to 751±19 g m^{-2}) and F-first (ranging from 389±20 g m^{-2} to 570±44 g m^{-2}). The L-first treatment with the densities 1.5 g m^{-2}, 2.5 g m^{-2} and 5 g m^{-2} being on average 25.3%, 30.5%, 27.8% more productive than their respective controls in the six week interval treatment. The treatments with a three-week sowing interval and L-first were on average 4.9% more productive in the 1.5 g m^{-2} density and 4.0% more productive within the 5 g m^{-2} density than their respective control, whereas there was no increase in productivity at 2.5 g m^{-2} (Fig. 2). Our experimental design was fully balanced except for the controls, i.e. all PFTs sown at same time, where we had three control replicates but one replicate was harvested at first harvesting date for the three-week sowing interval and the

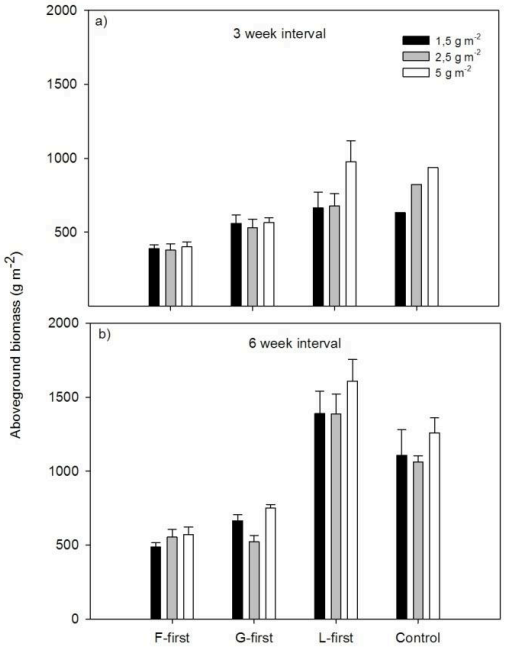

Figure 2. Sowing legumes first (L-first) produced the highest aboveground biomass, especially in the six-week interval treatment. Density had weaker effects on biomass than the priority effect treatment or sowing interval. Data show mean aboveground biomass (±1 SE) in the priority treatment separated into the three density levels. This is shown per sowing interval treatment with panel a) describing the three-week sowing interval and panel b) the six- week sowing interval treatment. For the priority effects treatment F, G and L-first = forbs-, grasses- and legumes-first. Replicates are n = 4 for main treatments and n = 2 or 1 for the controls in the six-week interval and three-week interval respectively.
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remaining two at the second harvesting date for the six-week sowing interval. This made sure that we allowed each plant community the same amount of time to develop after sowing. Taking this into account, the increase in productivity of the L-first group over that of the controls seemed not to be different between the three-week interval treatments (but no replication) but was significant in the six-week interval ($F_{(3, 38)} = 74.847$, $P < 0.0001$).

Interactions were found between the factors priority effect and sowing interval ($F_{(3, 57)} = 7.466$, $P < 0.0001$, see Table. 1). As a consequence, a Type I ANOVA was performed showing that irrespective of the sequence in which the other factors were fitted to the model, priority effect remained significant ($F_{(3, 57)} = 67.935$, $P < 0.0001$).

Species & PFT Relative Abundances

The PFT sown first always dominated the functional composition of the plant community. Nevertheless, there was a clear difference between treatments with a three-week interval and a

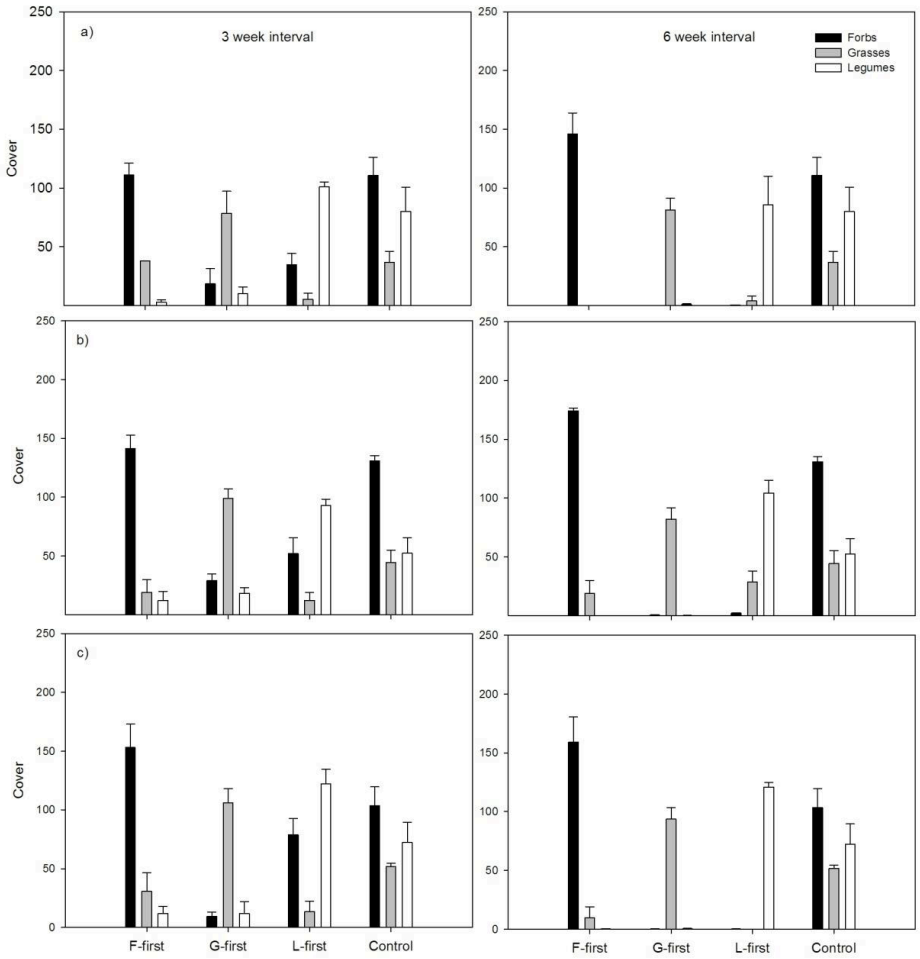

Figure 3. Effects of early sowing of one PFT (F, G and L-first = forbs-, grasses- and legumes-first) on the functional composition of communities in pots. Relative cover of PFTs (forbs, grasses, legumes) in pots were derived from individual species cover values summed and are depicted separately for each of the three densities: (a) 1.5 g m^{-2}, (b) 2.5 g m^{-2} and (c) 5 g m^{-2} for both sowing intervals (three and six weeks, in vertical columns) from vegetation assessments at peak biomass development. The data show mean values (±1 SE); n = 4 for all treatments (except for controls where n = 3).
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six-week interval. For treatments with a six-week sowing interval the relative abundance of the PFT sown first was nearly always > 90% except for one case (L-first treatment with a density of 2.5 g m^{-2} (78.6%)). The three-week interval treatment showed a more balanced relative abundance of PFTs. Priority effects on PFT abundance were consistent among the three density levels, favouring the PFT sown first with 73–84% relative abundance of forbs when forbs were sown first, 67–83% for grasses when grasses were sown first and 59–72% for legumes when legumes were sown first (Fig.3). Control treatments with simultaneous sowing showed forbs with relative abundances between 44–59%, grasses between 15–23% and legumes between 22–41%. Highest relative abundances in each PE-group were always in highest densities except for the L-first treatment where highest PFT relative abundance (72%) was at 1.5 g m^{-2}.

Within the L-first treatment subsequently-sown PFTs (grasses and forbs) were able to establish themselves better alongside the PFT sown first (legumes) compared to the other treatments (F- or

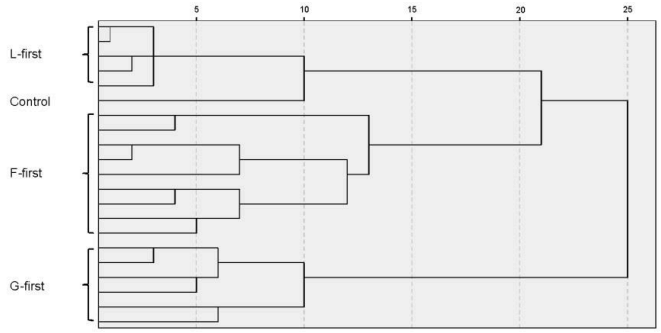

Figure 4. Effect of experimental treatments on the similarity of the resulting communities. Dendrogramm showing between group linkages for all replicates of the treatment groups: density (1.5, 2.5 and 5 g m^{-2}; sowing interval (3- and 6- week) and the priority effects treatment F, G and L-first = forbs-, grasses- and legumes-first as a result of a cluster analysis performed on data for relative individual species cover of every single pot in June 2011. As a measure of distance between groups Pearsons correlation coefficient was taken.
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G-first) where subsequently sown PFTs were suppressed (Fig. 3). This effect was stronger in the shorter sowing interval of three-weeks. Community development was clearly affected by the priority treatment and communities having the same starting PFT were more similar than those with different starting PFTs. A cluster analysis based on data on single species cover from vegetation assessments revealed three main groups in terms of species composition, and that these groups were mainly influenced by the starting PFTs. Most differences were found between communities with G-first and the rest, followed by a separation of the F-first group and a combined L-first and control group (Fig. 4).

Change in Soil Chemistry

Soil phosphate, nitrate and potassium were depleted by the end of the experiment when compared to values from the beginning of the experiment (T-test $P<0.05$; for details see Supporting Information, Table S4: Results of the T-test as a comparison of soil nutrient levels at the beginning and at the end of the experiment). In comparison to the nutrient content of soil samples collected at time zero, C/N ratios were higher at the end of the experiment than at the beginning ($t_{(82)} = 2.773$, $P<0.05$). However, no experimental treatments had any significant effects on the measured soil variables (for details see Supporting Information, Table S3: ANOVA performed on the effect of PE-treatment on soil variables).

Effect of Density on Aboveground Productivity

Density had a significant effect on aboveground productivity (Fig. 2; $F_{(2,\ 57)} = 5.567$, $P<0.05$) with a slightly higher productivity for the higher density levels. Nevertheless within the PE- and sowing interval treatments only a few treatments showed differences in aboveground biomass as a consequence of varying density.

For the L-first treatments and the three-week sowing interval, contrasts showed that the 5 g m^{-2} treatment had a significantly higher aboveground biomass compared to the lower sowing densities ($t_{(9)} = 2.143$, $P<0.05$). Within the G-first treatment similar biomass yields were found in all densities in treatments with a three-week interval but not in the six-week interval. Here the 2.5 g m^{-2} group was significantly less productive ($t_{(9)} = -3.975$, $P<0.005$) than the other two density levels.

The density treatment also influenced the number of individuals per pot, insofar as with increasing density the mean number of individuals increased across all treatments. Treatments with a sowing density of 1.5 g m^{-2} were having the lowest mean number of individuals ($t_{(66)} = 4.200$, $P<0.001$) and treatments with a sowing density of 5 g m^{-2} were having significantly higher numbers of individuals ($t_{(66)} = 2.841$, $P<0.005$) for both sowing interval treatments (Fig.5).

The influence of the priority effect-treatment was also visible in terms of numbers of individuals and showed the same trend for both sowing intervals. Treatments with a six-week interval had fewer individuals in each density level than in the three-week interval treatment ($t_{(67)} = 3.846$, $P<0.001$; Fig. 5).

Sowing Interval Effects

The effect of sowing interval on aboveground productivity between sowing of the first- and subsequent PFTs was significant (Fig. 2; $F_{(0.399)} = 59.313$, $P<0.0001$), with a sowing interval of six weeks showing increased productivity across all levels of the density treatment compared to the three-week interval. On average all treatments within the six-week interval group were more productive than the groups with a three-week sowing interval. The most pronounced difference in aboveground biomass was visible for the L-first treatment. In comparison (all sowing densities together) the L-first treatment with a six-week interval had 89% more biomass compared to the three-week interval group. The other PE groups for F- and G-first showed 38% and 16% increase in aboveground biomass respectively in comparison to the treatments with a three-week interval. Simultaneously sown controls were on average 62% more productive in the six-week interval group compared to the three-week interval control treatments.

The sowing interval also had strong effects on overall number of individuals per pot ($t_{(76)} = 3.588$, $P>0.005$; Fig. 5) and the overall plant species richness ($t_{(76)} = 4.376$, $P>0.001$) with lower individual and species numbers in the six week sowing interval.

Discussion

Our study found that priority effects of order of arrival at plant functional level had a substantial effect on aboveground productivity of sown communities, with L-first treatment being more

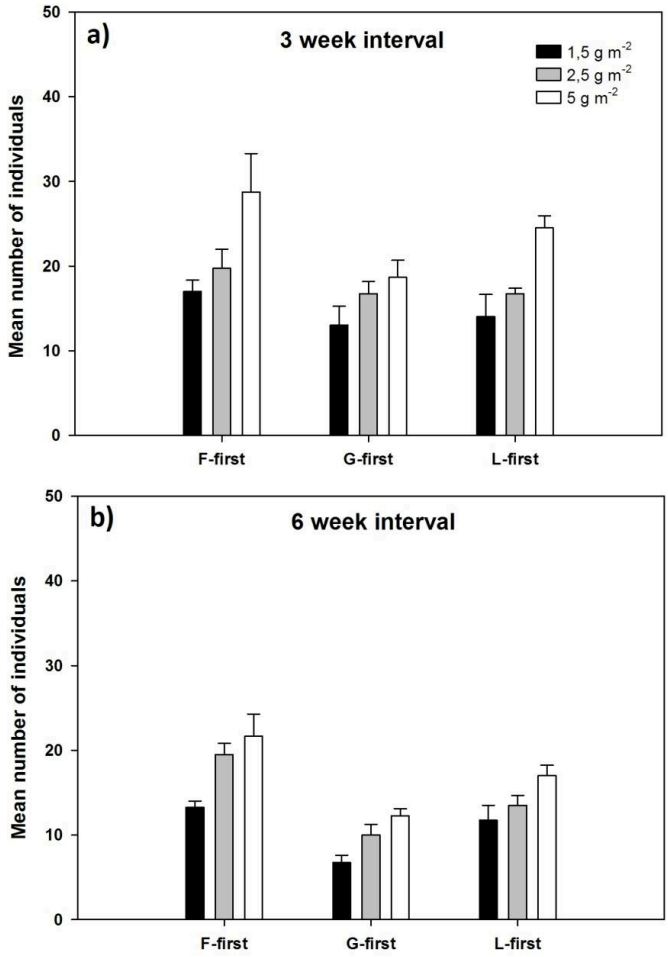

Figure 5. Effects of density and sowing interval on number of plant individuals per pot. For the priority effects treatment (PE) F, G and L-first = forbs-, grasses- and legumes-first. Values are mean number of plant individuals per pot with the PFT sown first on the x-axis and for all three densities for both groups sown with a three week interval (a) and a six week interval (b) between sowing of the first and remaining PFTs. (n = 4; ±1 SE).
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productive than controls sown at the same time or grass or forb species sown first (Figures. 2). These results (see Figure 4) confirm findings of Körner et al. (2008) and Ejrneas et al. (2006) in that the order of arrival of functionally different groups was critical to the development of their experimental communities resulting in clusters of different floristic distances to one another. In our study this is clearly reflected by the cluster analysis (Figure 4) on the basis of species' occurrence and relative abundances and the correlation to the functional composition of the resulting community. This analysis shows that the strong separation of communities was dependant on the starting PFTs which underlines the importance of priority effects in influencing the assembly process as found in a number of other studies [10,11,13]. Körner et al. [10] found that in terms of biomass production and final functional group composition after two growing seasons the L-first treatments and simultaneously sown controls were the most similar. Our experiment confirms this, even if our study ran for a shorter length of time and with a different species pool.

Our cover data confirm the aboveground biomass data in that in the L-first treatment, the functional groups were present in more balanced abundances than when grasses or forbs were sown first. Nevertheless, in the L-first treatment legumes contributed more to the overall community biomass than the other starting PFTs when they were sown first. L-first treatments were more productive than the other priority treatments irrespective of the sowing interval or sowing density, despite the fact that forbs were very dominant in relative cover and mean number of individuals. This suggests that legumes were better able to get a head-start and be productive before the later groups arrived even though species richness of the communities was rather modulated by the sowing interval (lower species richness when sowing interval was bigger) than by the identity of the species sown first, their traits allowed for better subsequent establishment of non-legume PFTs. In our experience legume species often do compete well and grow quickly in initial stages of experiments, as well as allowing for N facilitation with neighbours. Although legumes may not arrive earlier than other functional groups in naturally assembling communities, in ecological restoration we often wish to direct succession onto a desirable trajectory [34].

It seems that sowing legumes first led to asymmetric competition and fast growth of legumes [35] but at the same time more functional complementarity occurred between legumes and the other PFTs. A possible mechanism is the smaller rooting system (root mass fraction) of legumes if they are actively fixing atmospheric nitrogen, such that subsequent PFTs have more opportunities for both root space and nutrient foraging (also known as N sparing, [36]) and hence overall productivity is stimulated. Over a longer time span and under field conditions however, N facilitation (whereby the neighbours of N_2-fixers profit from legume-fixed N) may also cause higher productivity [37]. In this experiment treatments did not affect soil chemistry significantly even if C/N ratios changed from the beginning to the end of the experiment.

A likely explanation for the strong presence of forbs (at least considering cover and species numbers; Figures. 3&5) could be that forbs were overrepresented in species number right from the beginning (compared to the other PFTs there were 14 species sown within this functional group and only 7 for each legumes and grasses) to reflect the species and PFT composition common for restored grasslands in central Europe. But thus the F-first treatment had the lowest aboveground productivity, at least for our study no positive relationship between cover and productivity could be confirmed in this case (compare [27]).

We could not confirm the hypothesis that the longer the sowing interval the lower the aboveground productivity of the system will be. We hypothesised also that this would be because of stronger asymmetric competition between PFTs when early arrivers get a head-start and very little complementarity between PFTs can occur. What we found instead was that communities with a six-week sowing interval were more productive than those with a three-week interval (Figure. 2) despite the data showing higher mean species numbers (and also a higher species richness) in pots with a three-week sowing interval. A likely explanation would be that the starting PFT in the six-week interval group had three weeks longer to establish itself and grow than the three-week interval group. While the timespan for the two interval groups was the same after the second sowing occurred, meaning that for the two subsequently sown PFTs in every treatment the time allowed for growing was similar, the PFT sown first had 3 weeks more time to develop within the six-week interval. In general, later arriving PFTs contributed less towards community biomass as a consequence of the PE treatment and this makes sense since competitive advantage of the PFT sown first and thus asymmetric competition is part of the expected priority effect. Kardol et al. (2013) postulated that a priority-driven competitive advantage of early arriving species over later arriving species affected the probability of species coexistence and led to reduced species richness through competitive exclusion. This corresponds to our findings as we could also show a reduced number of individuals and lower plant species richness in the six week interval groups compared to the three week interval groups indicating the suppression of later arriving species by the PFTs sown first.

This could also be seen by looking at relative PFT contributions for the three- and the six-week interval (Fig. 3) where the six-week interval treatment was always particularly dominant without substantial contribution by the later sown functional groups species. We consider the starting PFT had a competitive advantage of arriving first and having better access to resources (especially light) before the competition with later arriving species occurred. As a result, niche space was likely filled more efficiently by the PFT sown first in the longer sowing interval treatments resulting in lower resource availability for later arriving plants as observed in other systems [38]. For a sowing interval of six weeks we observed an intensified dominance of the PFT sown first which was almost always above 90% in relative abundance at peak biomass whereas in the three-week interval, later sown PFTs were still able to compete and sustain a higher proportion within the communities.

Our hypothesis stated that because of the law of constant yield, sowing different seed densities will result in higher individual numbers at higher sowing densities, but overall aboveground productivity will remain the same across the different densities. Aboveground productivity did not differ across the density treatment but at the same time mean number of individuals per pot were significantly higher in treatments with higher sowing densities (Fig. 5). However, this difference did not strongly affect aboveground biomass and this suggests that soil nutrients were fully exploited by the community independent of how many individuals were present. As a consequence, higher sowing densities did not result in higher overall aboveground biomass, possibly because each individual was not able to be as productive as in lower density treatments, which is consistent with the law of constant yield [25]. It seems that the duration of our experiment was long enough for the law of constant yield to take effect.

Conclusion

The influence of assembly history on aboveground productivity was much stronger than sowing density or sowing interval (see Table 1 showing different effect sizes of factors). PE treatments led to the development of differently structured plant communities in terms of plant functional composition and dominance structure (Figs. 3&4). In natural succession plants often follow a sequence in which certain species establish and represent the community at a certain time point. This is often controlled by the local species pool and the availability of suitable environmental conditions for establishment. In our experiment both determinants were excluded (as often done in restoration practices) in the setup and thus the observed priority effect is of purely artificial nature. An important aspect of the priority effect was that the PFT sown first had significant effects on further functional composition with strong dominance of the early arriving PFT in the community. Although a larger sowing interval led to higher asymmetric competition we found evidence for complementarity between PFTs in the three-week interval treatment. In the latter, the cover of later arriving PFTs was larger than for other treatments when

legumes were sown first, suggesting that the optimal combination of functional groups would be sowing legumes first but making sure the sowing interval was not too long to enable the plants to achieve full maturity and thus have negative impacts on newcomers. Our results indicate that priority effects affect community development and function and that the severity of this impact seems to be much more driven by the question "Who comes first (and what is their function)?" than by questions like "when?" or "how many?". A possible application for our results can be found within the fields of restoration or agricultural practise when it comes to restoring ecosystem services or to increase productivity in low input high diversity systems [39,40]. To what extend we are able to set direction and to influence the development of plant communities via priority effects and their potential to create alternative stable states within plant communities is still to be addressed. So far to our knowledge no field experiments have tested these priority effects of functional group arrival time on community assembly and this would include a longer term and of course larger-scale assessment of priority effects on structure and function of communities. We are currently addressing this in a field experiment with the same kind of PFT-first treatments as in this pot experiment, where that we also find priority effects of sowing legumes early, even if one allows other species to invade aside from the sown species. Our study nevertheless confirms previous concepts of legumes as keystone species within N-limited grassland habitats, since the legumes seemed to have the ability to dominate at the same time as interacting with other groups in a complementary way [29,41]. Other studies have proposed asymmetric competition and plant-soil feedback effects as possible mechanisms behind priority effects (e.g. Grman and Suding 2010). Our study emphasises the need to also consider N facilitation effects of legumes as a driver of priority effects.

Supporting Information

Table S1 Plant species per functional group with respective seed mass per pot.
(DOCX)

Table S2 Results of soil analysis at the beginning of the experiment.
(DOCX)

Table S3 ANOVA performed on the effect of PE-treatment on soil variables.
(DOCX)

Table S4 Results of the T-test as a comparison of soil nutrient levels at the beginning and at the end of the experiment.
(DOCX)

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Author Contributions

Conceived and designed the experiments: VMT PvG NDJ CB UR CP. Performed the experiments: PvG CP VMT. Analyzed the data: PvG VMT. Contributed reagents/materials/analysis tools: VMT CB UR. Wrote the paper: VMT PvG.

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Manuscript 4

Intraspecific phenotypic trait variation in *Plantago lanceolata* in field and mesocosm biodiversity experiments

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Intraspecific phenotypic trait variation in *Plantago lanceolata* in field and mesocosm biodiversity experiments

Running title: Trait variation in *Plantago lanceolata*

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Abstract
Trait-based approaches are increasingly used to study mechanisms controlling the coexistence of plant species, particularly across environmental gradients. Intraspecific (as opposed to interspecific) trait variation has received considerably less focus in this regard despite its importance for understanding species distribution and adaptation.
Here, we investigate intraspecific phenotypic trait variation (used as a proxy for the amount of phenotypic plasticity) of the grassland forb species *Plantago lanceolata* under different environmental conditions. We measured functional traits of *Plantago lanceolata* (population mean traits) growing on different substrates and with different neighbors under both mesocosm and field conditions over time. We also compared the trait variability (the coefficient of variation, CV) within experiments and between experiments (mesocosm vs. field). In mesocosms, traits responded as expected much more to soil fertility (substrate type) than to neighboring species, but trait values in more fertile and less fertile substrates converged over time. In field settings soil fertility had less influence on trait differences, however some leaf traits responded to legume cover. We found high trait variability in the low fertility substrate and much less variability in the more fertile soils in the mesocosms. The tested conditions had more influence on differences in mean trait values and trait variability in the mesocosms than under field settings. Traits were more stable under different environmental conditions in field settings. This suggests, as hypothesized, that less favorable, more stressful sites may favor higher phenotypic plasticity. Multiple concurrent factors as found under field conditions can lead to a more conservative phenotypic range. Substrate type influenced trait variation more than legume neighborhood under controlled conditions, but interestingly the opposite was true in the field. Further studies should investigate whether these findings hold true for other herbaceous species, as this could have important implications for interpolating between lab and field studies or across environmental gradients.

Introduction

Trait-based approaches are useful for addressing and understanding the mechanisms controlling the coexistence of plant species and how plant communities are structured across environmental gradients (Lavorel and Garnier, 2002; McGill et al., 2006; Ackerly and Cornwell, 2007; Violle et al., 2007; Webb et al., 2010; Violle et al., 2012). Plant traits can be addressed in two different ways: variation in traits among (interspecific/between) and within species (intraspecific). Both intraspecific and interspecific variation can influence community structure (Ackerly, 2003). Intraspecific trait variation, which can be also differentiated in within and between populations of the same species (Schlichting, 1986; Lemke et al., 2015), may arise from genetic variation or phenotypic plasticity, which is the ability of a single genotype to produce different phenotypes in reaction to environment (Schlichting, 1986; Sultan, 1995; Miner et al., 2005). Plasticity enables plant populations to respond to global changes in the short-term and through the maintenance of genetic variation also promotes their long-term persistence (Matesanz et al., 2010; Lemke et al., 2012).

Plant functional traits are defined as morphological, physiological and phenological characteristics that directly or indirectly affect individual performance and fitness of a species (McGill et al., 2006; Violle et al., 2007). Intraspecific trait variation can be large in response to abiotic and biotic constraints (Albert et al., 2010 b; Violle et al., 2007; Jung et al., 2010; Mitchell and Bakker, 2014 b). Most community assembly studies, where trait based approaches have been used, ignore the role of intraspecific variation in trait values compared with interspecific variation (Albert et al., 2010 a; Violle et al., 2012). Recent studies have however shown how important intraspecific variation can be for the maintenance of species coexistence and in determining functional community composition (Albert et al., 2010 a and b; Messier et al., 2010; Albert et al., 2011 and 2012; Violle et al., 2012). Albert et al. (2012) found that intraspecific trait variation may strongly alter the quantification of functional diversity and the detection of ecological patterns in a changing environment. It has been found that intraspecific trait variation promotes species coexistence, by enabling species to pass through both abiotic and biotic filters and hence persist at a site (Fattorini and Halle, 2004; Jung et al., 2010). If a species has high intraspecific trait variation it will be more likely to persist in a broad range of environments and so be more resilient within a community in the face of change (Andrade et al., 2014).

Intraspecific trait variation can occur at different levels (Albert et al., 2010 a and b): “as differences between mean traits of populations living under contrasting environmental conditions, as the differences between individuals of a population (variability) under one particular set of environmental conditions, or as differences in trait values within individuals (e.g. at leaf level)”.

Most new approaches measure intraspecific trait variation either under different climatic scenarios, along environmental gradients (flooding, drought), in relation to surrounding biodiversity, during community assembly or between different regions (between different populations of the same species) (including measurements of environmental conditions) (Jung et al., 2010; Beierkuhnlein et al., 2011, Lemke et al., 2012; Wellstein et al., 2013; Jung et al., 2014; Lipowski et al., 2015). Very few studies have compared intraspecific trait variation across different environmental conditions and over time during community assembly.

As such we know very little about how intraspecific trait variation is general structured both spatially and temporally (Albert et al., 2010 b). In terms of how intraspecific trait variation is structured by time, our study focuses on this a key component of our analysis. We focus on changes in traits of adult individuals of this plant species over time, not related specifically to

phenology (we did not measure flowering for example) or seasonal changes or succession but related to any changes that occurred due to a range of drivers over the time frame studied (up to three growing seasons). Our study has the advantage that various environmental and experimental factors are located in the same location, with the same weather (except for the Lindenhof Experiment) but that the treatment factors can have different effects over time, as we know from many previous biodiversity experiments. This allowed us to explicitly focus on how traits changed over time, knowing that the weather conditions were the same (in a common garden approach).

In addition to the paucity of studies focusing on within and between species trait variation, comparisons of plant trait variation within species when growing in the field compared with more controlled conditions are rare. Mitchell and Bakker (2014 a) recommend performing studies in greenhouse or common garden settings to tease out the factors influencing the observed differences in traits and variation. This should also allow us to understand better whether this variation is adaptive or beneficial. We expect that the wide range of factors that act simultaneously on plant performance in the field will lead to a defined conservation of traits under field conditions compared to more controlled conditions. This is because under more controlled conditions less factors influence growth and fitness (e.g. less herbivory, weather and plant-soil interactions) so that a plant species has to deal with less tradeoffs in relation to growth.

We chose *Plantago lanceolata* (hereafter called *P. lanceolata*) as a plant species with a broad niche and distribution, growing in central, northern and southern Europe (Database: Ecological Flora of the British Isles[1]). In addition, it is a very abundant species in mesotrophic grasslands of central and northern Europe and a frequently studied species in biodiversity-ecosystem functioning experiments (Spehn et al., 2002; Temperton et al., 2007; Mommer et al., 2010). As such the species' populations are exposed to a large range of climatic conditions, including deep winter frosts and dry periods. In consequence, it recovers swiftly from drought (Jentsch et al., 2011; Ravenscroft et al., 2014).

Presence of specific plant functional groups (e.g. N2-fixing legumes) can also influence traits of plant species. Temperton et al. (2007) studied positive effects of legumes on neighboring plants across a plant diversity gradient in a field experiment, with *P. lanceolata* as one of four phytometer species transplanted into every plot. They found that *P. lanceolata* benefitted from extra nitrogen (N) when growing near legumes but did not manage to translate this into higher aboveground biomass when growing in legume monoculture. In high-diversity communities *P. lanceolata* showed some positive response (Temperton et al., 2007). Equally, *P. lanceolata* experienced lower levels of N transfer from the legumes than other forb and grass species in the phytometers than *Knautia arvensis* or *Festuca pratensis*.

Evidently, *P. lanceolata* occupies a broad niche and has a wide distribution. It can adapt to a range of different environmental conditions by optimally allocating available resources to growth and fitness in different ways under different conditions. This may lead to trait convergence or trait dispersion (traits becoming increasingly different across different environments). If trait values are similar, then trait convergence has occurred in response to a wide range of factors in each environment, suggesting that a given plant species possesses the ability to optimize or restrict growth despite (or because of) a range of different tradeoffs affecting growth. If trait dispersion occurs then a species exhibits a wide variation in traits

[1] http://www.ecoflora.co.uk

across a range of abiotic and biotic conditions (including low stability in trait values, i.e. a high value for the coefficient of variation).

In this study we investigated the variation in aboveground traits of *P. lanceolata* across a range of environmental conditions (substrate type, neighborhood, experimental set-up and time). We define trait variation as an umbrella term for both the differences between trait means of populations across a range of environmental conditions/treatments as well as the variability of trait values. We define variability, in contrast as a measure of trait dispersion (relative amount of trait variation around the population mean e.g. the variability of trait values measured as the coefficient of variation).
This study aimed to describe intraspecific trait variation under various conditions both in the field and under semi-controlled conditions. To do this we used a set of different experiments (mainly) in the same location, experiencing the same weather conditions, the only differences were the lab to field or legume presence or soil/substrate type components. The main focus these experiments is not the main focus of this study, but the trait variation of *Plantago lanceolata*, which was present in all the experiments. For this reason, we present our data in such a form that one can see the overall variation in traits, without having to go into detailed assessment of the effects of treatments. The effects of different potential drivers of trait variation can then be compared in the tables and graphs.

We therefore asked the following question:
Is species' trait variation (mean trait differences and trait variability) similar under varying site conditions in P. *lanceolata*?

a. Do environmental conditions and treatments result in increased differences in mean trait values?
b. Does trait variability (its coefficient of variation, i.e. stability of traits) differ under the similar environmental conditions?
c. Does trait variability differ between controlled and field experiments?

We hypothesized that:

1) substrate type (soil fertility) will have a stronger effect on trait variation (mean trait differences and trait variability) in *P. lanceolata* than neighborhood or time. We formulated this hypothesis since it is well known that soil fertility and type has strong effects on plant growth and hence also various functional traits, whereas effects of legume neighborhood are not always as strong or clear as effects of soils. In addition, barely any studies focus on how traits change over time and adult plants growing under similar environmental conditions, focussing instead on effects of spatial or environmental drivers. Thus our study allows us to investigate new aspects of trait variation.
2) trait variation (mean trait differences and trait variability) in *P. lanceolata* under our tested conditions will be lower under field conditions compared to when growing in mesocosms since the larger range of influences on fitness in the field will select for a more conservative phenotypic plasticity.

Materials and Methods

To ensure comparability of *P. lanceolata* individuals across the different treatments conditions, we only used seeds from one particular region of Germany (Nordrhein-Westphalen), i.e. the region in which we performed the study. All seeds were obtained from the wild plant seed company Rieger Hofmann GmbH (Blaufelden, Germany). One exception to this rule was the seeds used for the Lindenhof experiment, which were obtained from various sources already in the mid-90ies.

Design of the Mesocosm Experiment

In spring 2009 (May) we established a Mesocosm Experiment for analyzing the effects of two different substrates (low and high nutrient content) as well as local neighborhood (monoculture vs. mixtures; species identity of neighbors) on traits of four selected species over a period of three years. In this study we focus only on *P. lanceolata* plant traits and their response to different environments.

The Mesocosm Experiment was set up outdoors next to the Habitat Garden (a community assembly field experiment, see Plückers et al., 2013 b) on the campus of the Forschungszentrum Jülich, Germany (6° 22'0''E, 50° 56'0''N). The Experiment consisted of 42 mesocosms which were randomized each with a volumetric capacity of 58 litres (mesocosms had a tapered shape, with a square base of 0.4 x 0.4 m in size see photo in Table 1). We used two different substrates (n = 21) simulating substrates and soils use in the adjacent Habitat Garden Experiment (one very low nutrient and one high/medium fertile soil; see Plückers et al. 2013 b). The nutrient-poor substrate consisted of sand (grain size 0.7 - 1.4 mm) mixed with two tents greenhouse potting soil producing a very low-nutrient substrate, at pH = 6.0 $\pm$ 0.1, ($\%C_{total}$ = 0.57 $\pm$ 0.17, $\%N_{total}$ < 0.02). The nutrient-rich substrate consisted of six tenth potting soil of moderate /high nutrient availability (pH = 6.15 $\pm$ 0.05; $\%C_{total}$ = 3.56 $\pm$ 0.12, $\%N_{total}$ = 0.13 $\pm$ 0.01). Over time, carbon content increased for both substrates (when comparing time zero (2009) to later time-points (2010 and 2011) with species present) and then stayed stable, whereas nitrogen content and pH (6-7) were relatively stable over the whole experimental period time.

Species selected for mixtures were chosen based on their dominance in the Habitat Garden assembly plots: *Plantago lanceolata*, *Hypochaeris radicata*, *Trifolium repens* and *Lotus corniculatus* (seeds from Rieger Hofmann GmbH Blaufelden, Germany). In August 2009 we sowed seven different neighborhoods at a seed density of 4g/m^2. Four monocultures (n = 3) and three combinations of two species combinations (*Plantago lanceolata* with *Lotus corniculatus* or with *Hypochaeris radicata*; and *Hypochaeris radicata* with *Trifolium repens*) (n = 3). The experiment was fenced off to reduce confounding factors such as herbivory by large mammals. Mesocosms stayed outdoors exposed to ambient environmental conditions during the whole experimental period from summer 2009 until 2012, and were not fertilized, only additionally watered in May 2011 with equal volumes of water per mesocosm because of less rainfall and most sun hours in this month. Some of the nutrient-poor mesocosms had to be re-sown in 2010 because of poor plant performance. Non-target species were weeded-out regularly in all years.

Design of the field experiments (Habitat Garden and Lindenhof)

We measured traits of *P. lanceolata* in two field experiments where it was surrounded by a large number of neighboring species. The Habitat Garden Experiment was designed to analyze grassland community assembly depending on different starting diversity (Plückers et al., 2013 a and b). This experiment was established on the campus of the Forschungszentrum Jülich in autumn 2007 with two different grassland habitats (dry acidic and mesic grassland, n = 6 per grassland type). The nutrient-poor sandy substrate for the dry acidic grassland consisted of sand (grain size 0.7 - 1.4 mm) mixed with one tenth potting soils (with very low nutrient availability, $\%C_{total}$ = 0.017 $\pm$ 0.002, $\%N_{total}$ = 0.005$\pm$ 0.0001 (time zero)). The nutrient-rich substrate for the mesic grassland consisted of an ex-arable soil type of the region Heinsberg, Germany ($\%C_{total}$ = 1.039 $\pm$ 0.063, $\%N_{total}$ = 0.096 $\pm$ 0.004 (March 2008)). In December 2007 we sowed three differently diverse seed mixtures (Rieger Hofmann GmbH Blaufelden, Germany) at a density of 4 g/m^2 on the 12 plots (randomized, each 2 x 2 m in size and separated by 50 cm paths sown with a non-clonal grass species).

Table 1. Overview of all traits measured across a range of different conditions (experimental set-up, substrate type, neighborhood and time). The letter **x** denotes when a trait was measured in a particular experiment. S2 and S7 relate to differently diverse sown seed mixtures, with either two or seven grass species and a total species richness of 27 and 39.

Traits	Conditions			
	Experimental set-up	Semi-controlled conditions	Field conditions	
		Mesocosms	Habitat Garden	Lindenhof
	Substrate type	Nutrient-poor and -rich	Nutrient-poor and -rich	Nutrient-rich
	Neighborhood	1 and 2 species (with a legume forb species or with a non-legume forb species	S2 and S7 (only nutrient-poor)	1, 2, 4, 8, 16 species and free sucession from 2009
	Time	2009-2011	2010 and 2011	1996-1998 and 2009-2011
Leaf dry weight		X	X	-
Leaf area		X	X	-
SLA		X	X	-
Leaf nitrogen		X	X	-
Leaf chlorophyll *a*		X	X	-
Plant height		X	-	-
Aboveground biomass		X	-	X
				

The dry acidic grassland plots were initially sown with two different diversity levels (n = 3 per sowing treatment S2 and S7) whereas the mesic plots (M) were sown with the same diversity of species at start (and removal experiments preformed at a later date; *P. lanceolata* was in the seed mixture). S2 consisted of 2 grass and 25 forb species (one of which was a legume), S7 consisted of 7 grass and 32 forb species (four of which were legumes, *P. lanceolata* was included) and M consisted of 11 grass and 23 forb species (two of which were legumes, *P. lanceolata* was included). The plots were not fertilized but were mown once a year in August in the dry acid grassland, twice a year in June and August in the mesic grassland plots according to typical mowing regimes for such grasslands in Central Europe. The nutrient-rich substrate had a higher carbon and nitrogen content than the nutrient-poor substrate. Over time, carbon and nitrogen content increased from time zero (2007) to 2010 and then decreased for both substrates. pH remained stable between 6-7 over the three-year experimental period.

The second field experiment where *P. lanceolata* traits were measured was the EVENT 5 experiment at the "Lindenhof" site in Bayreuth, Germany. This experiment is located next to the environmental station Lindenhof near Bayreuth (49°55'N, 11°35'E, 355 m altitude). The original experimental layout was part of the pan-European BIODEPTH biodiversity ecosystem functioning experiments (Hector et al., 1999). The aim of this study was to investigate the effects of plant diversity on ecosystem functioning; after 3 years weeding was ceased and the plots were allowed to go through natural assembly. At the site in Bayreuth, the experiment consisted of 64 plots (random block design, each 2 x 2 m in size). The soil was a loamy to sandy stagnic gleysol (in 1996 pH = 5.65 $\pm$ 0.2, $\%C_{total}$ = 0.78 $\pm$ 0.06, $\%N_{total}$ = 0.08$\pm$ 0.01; in 2002, % C_{total} = 0.77 $\pm$ 0.1, $\%N_{total}$ = 0.13$\pm$ 0.01) (Scherer-Lorenzen, 2000). A gradient of species and functional diversity was created by sowing different species mixtures with 16 (n = 6), 8 (n = 10), 4 (n = 10), 2 (n = 14) or one species (n = 20) at a total seeding density of 2000 viable seeds/m^2. Four plots were left bare as non-vegetated controls. Each diversity level was replicated with different mixtures and each mixture was repeated in two adjacent blocks (block A n = 32 and block B n = 32) so that each exact mixture was replicated twice. In the years 1996-1999 non-target species were weeded to hold species composition constant. After final weeding in 1999, natural succession was allowed to take place. The plots were not fertilized but were mown twice a year (in June and September according to typical mowing regimes for such grasslands in Central Europe). For more details see Neßhöver (2005) and Kreyling et al. (2011). In our study we were only interested in the years 1996-1998 and 2009-2011 and only in the plots where *P. lanceolata* was sown (in monoculture; 2, 4, 8 and 16 species mixture). This gave a total of 10 plots and two replicates for each mixture where *P. lanceolata* aboveground biomass was measured on a regular basis, providing data with which we could compare the biomass variability of *P. lanceolata* in the Mesocosm Experiment. We used the years from 1996-1998 to have a direct comparison of constant species diversity in the field compared to the mesocosms. We also took the years from 2009-2011 to have an additional comparison when free succession was allowed (and weeding ceased) allowing a comparison of traits when surrounded by high species diversity vs. constant species diversity in the mesocosms during the same time period (2009-2011).

We are aware that these three experiments (Mesocosm, Habitat Garden and Lindenhof) were not explicitly set up to be directly compared with one another, in the sense that the exact treatments and neighborhoods do differ somewhat. However, they do provide a useful platform where *P. lanceolata* was growing under either field conditions (in two different field experiments addressing issues of biodiversity and assembly) and semi-controlled conditions in the mesocosms (a competition experiment with one or two species, including legume effects). This platform allowed us to compare mean trait differences and the trait variability of

P. lanceolata across a broad range of environmental and biotic conditions. We thus compare the mean trait differences and the variability (coefficient of variation) between substrates and over time but we ignore neighborhood and species richness effects when it is not directly comparable between the different experiments.

Plant traits measured

Plant traits measured in *P. lanceolata* were leaf dry weight (leaf DW), leaf area, specific leaf area (SLA), leaf nitrogen (leaf N) and leaf chlorophyll *a*. Such traits were measured under semi-controlled conditions in the mesocosms and under field conditions in the Habitat Garden Experiment. Plant height was measured in the mesocosms. Aboveground biomass of *P. lanceolata* was measured in the mesocosms and under field conditions in the Lindenhof Experiment (see Table 1). To test for legume effects legume cover was assessed in both field experiments. Plant phenotypic traits in the mesocosms were measured twice a year (June/August and August/September) from 2009 till 2011 (except in 2009 only once a year in October during establishment of species). In the Habitat Garden Experiment traits were measured twice a year for the nutrient-rich plots (June/August) and once a year (August) for the nutrient-poor plots from 2010 till 2011. Aboveground biomass was measured in the Lindenhof Experiment twice a year (June/September, except during the initial year in 1996 to allow species to establish).

Trait measurements generally followed the protocol of Cornelissen et al. (2003). For leaf dry weight and leaf area we collected 2- 5 leaves per plot or mesocosm. Each leaf was cut directly above the soil surface/rosette. The fresh leaf was used for measuring leaf area (cm^2) with the LI-3100C Area Meter (LI-COR, Lincoln, NE, USA) and then oven dried at 70 °C for leaf dry mass measurement. Specific leaf area (SLA) in cm^2/g was calculated as the one-sided area of a fresh leaf divided by its oven-dry mass. Leaf nitrogen concentration was measured by grinding the oven-dried leaves to a homogenously fine powder and 80 mg sample was burned in an elemental analyzer (System: VarioelCube or Leco). Often there was not enough ground leaf material for analyzing leaf nitrogen content and leaf chlorophyll *a* in the nutrient-poor substrates for each replicate such that we used a pooled sample.

For plant height we measured the distance between the upper boundary of 4 -5 leaves and the ground level/rosette. Leaf chlorophyll *a* was analyzed by a chlorophyll extraction and a spectrophotometric measurement (Lichtenthaler, 1987; Lichtenthaler and Buschmann, 2001). Therefore 2-3 round cuts (1cm) of a fresh leaf were taken and frozen by -80°C for later extraction.

For aboveground biomass, all aboveground plant material per mesocosm was cut 1 cm above the soil surface, sorted into species and samples were dried at 70 °C to constant weight and then weighed (then scaled up to aboveground biomass g/m^2). For the Lindenhof Experiment aboveground species- specific biomass and biomass of functional groups (grasses, herbs, legumes, woody plants) (dry matter yield, g/m^2) were measured in two $0.1m^2$ quadrats (20 x 50 cm in size) cutting 5 centimeters above the soil surface to simulate traditional mowing devices within the central m^2 of each plot. Cover of species was estimated before mowing using a decimal scale based on Braun-Blanquet but modified by Londo (1976) for each species in whole plots. Total legume cover was the sum of all legume species cover per plot.

Climatic conditions

Annual precipitation at the Habitat Garden in the Forschungszentrum Jülich (measured at the local weather station) had a 50-year mean of 699 mm, and between 2009 and 2011 was 728 mm, 778 mm and 691 mm respectively. Mean annual temperature at the site was 9.9 °C (50

year average) and temperatures from 2009-2011 were 10.5 °C, 9.2 °C and 11.2 °C. There were no notable weather extremes (e.g. 100 year climate extremes) during the experimental time-period, except that mean annual temperature in 2011 was somewhat higher than the long-term mean. Annual precipitation at the Lindenhof site in Bayreuth was 712 mm (a 150-year average) and was 615 mm, 678 mm, 894 mm and 795 mm, 868 mm and 819 mm respectively between 1996 -1998 and in 2009 - 2011. Mean annual temperature was 8 °C (30 year- average), from 1996-1998 temperatures were 6.5 °C, 7.9 °C, 8.4 °C and from 2009-2011 8.6 °C, 7.4 °C and 8.9 °C. (measured at the climate station at the Ecological-Botanical-Garden which is approx.1 km from the BIODEPTH field site; Lüers et al., 2014).

Statistical analysis

We analysed the differences in mean values of phenotypic traits between populations of *P. lanceolata* related to environmental conditions. Additionally, we compared the trait variability/ relative amount of trait variation around the population mean across different environmental conditions within an experiment and between experiments. Both differences are proxies for trait variation, which we use as an indicator for the amount of phenotypic plasticity. Our overall data set consists of three different sub-data sets, the Mesocosm Experiment, the Habitat Garden Experiment and the Lindenhof Experiment (see Table 1).

Overall the Habitat Garden Experiment had a one factorial design testing effect of a nutrient-poor vs. a nutrient-rich substrate. The nutrient-poor substrate of the Habitat Garden Experiment was additionally tested for effects of different starting diversity with two levels of medium or high diversity. Response variables measured were leaf dry weight, leaf area, SLA, leaf chlorophyll, legume cover, leaf nitrogen and the coefficient of variation (CV) of leaf area, leaf DW and SLA. The Lindenhof Experiment is also a one factorial experiment testing effects of the factor sown diversity with 1, 2, 4, 8 and 16 species levels. Response variables measured were aboveground biomass and legume cover.

The Mesocosm Experiment was a two factorial experiment testing effects of the factor neighborhood (with 1 and 2 species) and the factor substrate (with a nutrient-poor and nutrient-rich type). Response variables measured were the same as in the Habitat Garden plus plant height, the CV of plant height in 2009 and aboveground biomass (see Table 1).

All statistical analyses were conducted in the Program R 3.1.2 (Team, R. C., 2014). Since we were interested in trait variation over time, we analyzed most of our data using Repeated Measures analysis. Because of missing data points we used mixed-effects models for repeated-measures ANOVA using the function „lme()“ (Pinheiro et al. 2014). Traits which were only measured at one time point were analyzed using a one or two-way ANOVA. We fitted one-way ANOVA using R function „aov()“ and for the two-way ANOVA models the function „lm()“ (Fox and Weisberg, 2011; Lawrence, 2013).

All data that did not conform to homogeneity of variance or normal distribution were transformed before analysis. Therefore, data were multiplied to the power of a specific factor, which was derived by a power-transformation test to achieve normality of the residuals and homogeneity of variance. This is a useful pre-processing technique and robust against outliers. Original means and standard errors are presented throughout in graphs and tables.
To analyze any traits difference within single years we used the two-sided student t-test ($p<0.05$).

Additionally, we calculated the coefficient of variation (CV), which is defined as the ratio of the standard deviation to the mean as a relative measure of phenotypic variability. This allows

us comparison of trait variability within and between different experiments, even when the means are different. The CV is often used in trait variation studies (Schlichting and Levin, 1984; Albert et al., 2011; Lemke et al., 2012; Wellstein et al., 2013). We calculated the CV for some of the traits by calculating it for each plot or mesocosm (for leaf area, leaf DW and SLA as well as plant height (enough single measurement points per plot or mesocosm)). Traits were measured on multiple individuals per plot / per mesocosm and the mean value for *P. lanceolata* for each plot then calculated (so we can get one mean and standard deviation value per plot) and then these values served as the datapoints for calculating CV means and standard deviations for particular treatments. Plots and mesocosms were replicated three times. This allowed for a statistical analysis of the CV data for these traits across replicates (one single CV value per replicate). Where we did not have data on variability of a trait within each replicate (aboveground biomass, leaf nitrogen and leaf chlorophyll *a* (one single measurement point per plot or mesocosm)) we calculated the CV for each trait using the mean trait values and the standard deviation over the replicates (thus measuring population level variability over the replicates e.g. one CV value per treatment/across all replicates). Thus here, the CV is the population trait variability under given environmental conditions of *P. lanceolata* measured over the replicates. For the Lindenhof Experiment we ignored the block design to have 2 replicates for each mixture, because every mixture was only sown once in every block.

Results

Mean trait values

Did soil fertility and species neighborhood affect mean trait values and how did time affect the outcome?

Soil fertility and time effects on mean trait values

In the Mesocosm Experiment, plant height increased over time within the growing season and was higher in the nutrient-rich substrate (Figure 1, Table 2A significant substrate and year effect). Furthermore, differences in plant height between substrates became smaller over the three years of the study, however in the 2009 growing season plant height in the nutrient-rich substrate had a higher increase than in the nutrient-poor substrate (Figure 1, Table 2A significant year X substrate interaction effect).

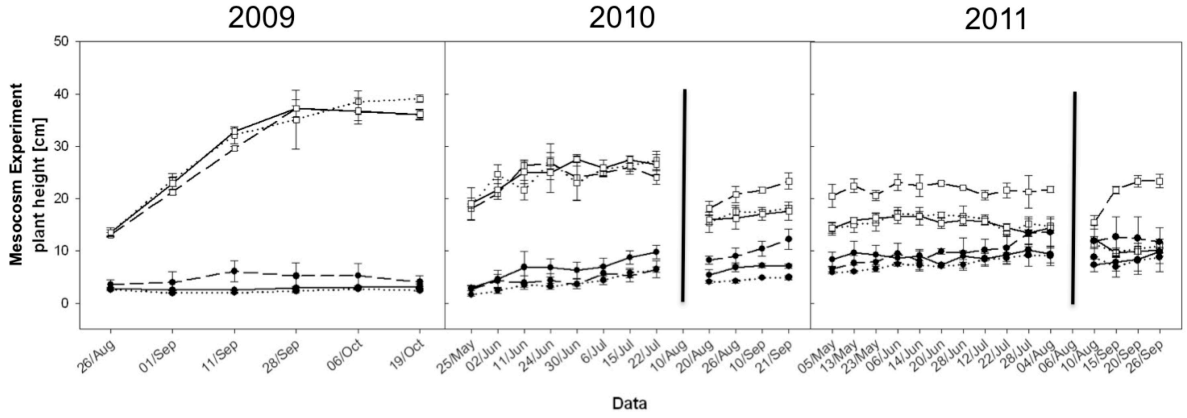

Figure 1. Mesocosm Experiment: The development of height of *P. lanceolata* over time (2009-2011) depending on substrate and neighborhood in the Mesocosm Experiment. For substrate treatment: nutrient-poor =circles, nutrient-rich = squares; for the neighborhood treatment: with a legume neighbor = dashed line, with a non-legume forb neighbor = dotted line, monoculture = black line. The black bold vertical line in 2010 and 2011 denotes the cutting of aboveground biomass. Values are means (± standard error of the mean, for significant differences see Table 2).

Leaf area, leaf dry weight, SLA and aboveground biomass of *P. lanceolata* decreased over time for the nutrient-rich substrate whereas these response variables were more stable for the nutrient-poor substrate over time, instead SLA also decreased for the nutrient-poor substrate (Figure 2 and 3, Table 2A significant year effect and significant interaction year x substrate effect, Supplementary Table 1). In nutrient-rich substrate leaf area, leaf dry weight and aboveground biomass were higher than in nutrient-poor substrate whereas SLA was higher in the nutrient-poor substrate (Figure 2 and 3A, Table 2A significant substrate effect, Supplementary Table 1). Leaf nitrogen was stable across substrates but over time it increased (Figure 2, Table 2A, calculated without 2009 because of missing data points for nutrient-poor substrate for this year).

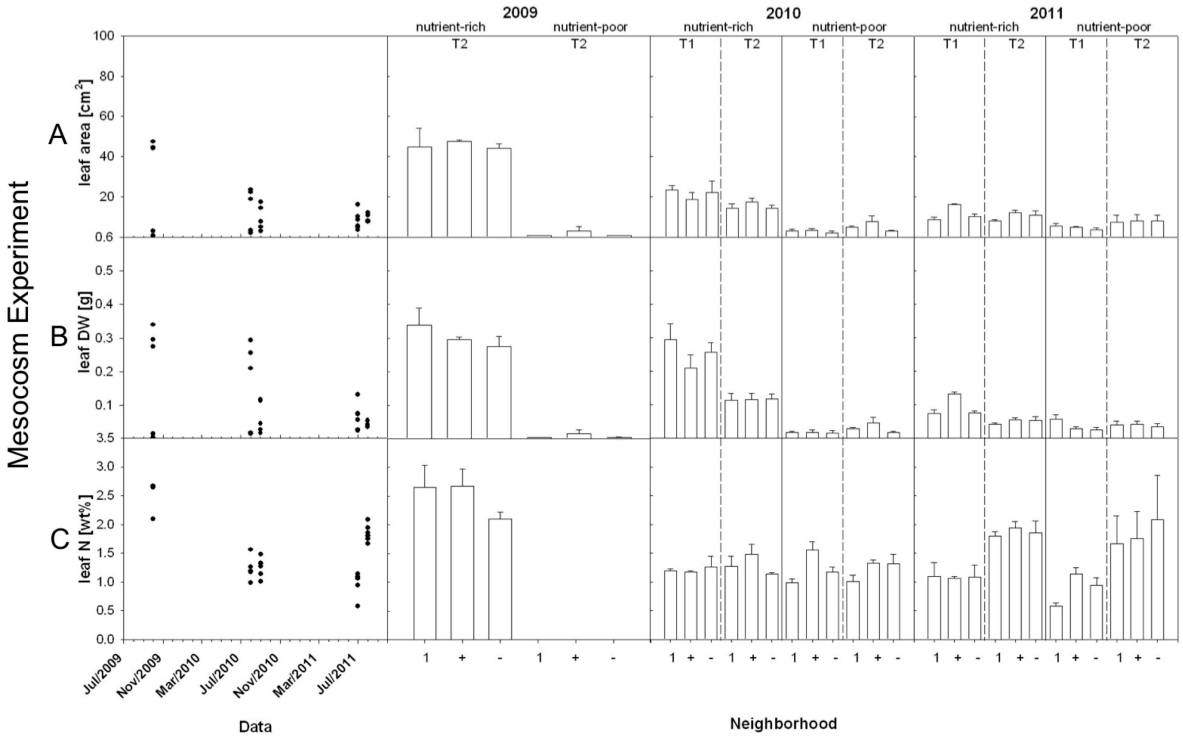

Figure 2. Mesocosm Experiment: Effect of substrate type and neighborhood on **(A)** leaf area, **(B)** leaf dry weight and **(C)** leaf nitrogen of *P. lanceolata* over time in the Mesocosm Experiment. Each first panel shows the spread of all the data over time independently of substrate and neighborhood. The following panels show the time periods from 2009 to 2011 separated by substrate type (nutrient-poor or –rich) and neighborhood (labels on x-axis: 1 = monoculture, for two species mixtures: + = with a legume neighbor, - = with a non-legume forb neighbor).T1 and T2 indicated the two measurements points in each year (August/September in 2010, July/August in 2011). Values are means (+ standard error of the mean,for significant differences see Table 2).

In the Habitat Garden Experiment (field conditions) we found smaller differences in mean trait values between substrate and time than in the Mesocosm Experiment: leaf area, leaf DW and SLA were similar between the soil fertility treatments and neighborhood treatments over time, only leaf area increased for both substrates over time (Figure 4A and B, Table 3A). In addition, there was a dramatic drop in both leaf area and leaf DW in the high fertility treatment between the first and the second measurement period (corresponding to time points just prior to the two mowing events of the growing season in mesotrophic grassland plots; see Figure 4A and B; significant t-test for 2010 p = 0.000175***(leaf area), significant t-test for 2010 and 2011 p = 0.01** and 0.005** (leaf dry weight)).

Table 2. Mesocosm Experiment: Results of Repeated Measures ANOVA testing the effects of substrate (low and high nutrients), neighborhood (monoculture, growing with a legume species or growing with a non-legume forb species), time (year) and their interaction on **(A)** mean trait values of *P. lanceolata* or **(B)** coefficient of variation (CV) of traits of *P. lanceolata* (trait variability) under semi-controlled conditions. Substrate and neighborhood effects show results of testing over the whole time span and the other factor, whereas Year effects describe how the effect of the substrate and neighborhood factors changed over the whole time. Each line represents a single analysis. The response variable of leaf chlorophyll *a* was tested by using a two-way ANOVA (since measured at only one time point).

Response variable	Factors													
	Substrate		Neighborhood		Year		Year X substrate		Substrate X neighborhood		Year X neighborhood		Year X neighborhood X substrate	
A	d.f.	P	d.f.	P	d.f.	P	d.f.	P	d.f.	P	d.f.	P	d.f.	P
Plant height 2009	1	<0.0001***	2	0.4270^{nsd}	5	<0.0001***	5	<0.0001***	2	0.1666^{nsd}	10	0.6954^{nsd}	10	0.3555^{nsd}
Plant height 2010 before cutting	1	<0.0001***	2	0.2983^{nsd}	7	<0.0001***	7	0.1579^{nsd}	2	0.5175^{nsd}	14	0.5406^{nsd}	14	0.3147^{nsd}
Plant height 2010 after cutting	1	<0.0001***	2	0.0002***	3	0.0010**	3	0.6666^{nsd}	2	0.0288*	6	0.5771^{nsd}	6	0.5919^{nsd}
Plant height 2011 before cutting	1	<0.0001***	2	0.0070**	11	0.0001***	11	<0.0001***	2	0.1360^{nsd}	22	0.3918^{nsd}	22	0.6183^{nsd}
Plant height 2011 after cutting	1	0.0119*	2	0.0011**	2	0.0218*	2	0.8346^{nsd}	2	0.0550^{nsd}	4	0.5800^{nsd}	4	0.1351^{nsd}
Leaf dry weight	1	<0.0001***	2	0.2311^{nsd}	4	<0.0001***	4	<0.0001***	2	0.6574^{nsd}	8	0.569^{nsd}	8	0.0621^{nsd}
Leaf area	1	<0.0001***	2	0.1131^{nsd}	4	0.0018**	4	<0.0001***	2	0.5554^{nsd}	8	0.8012^{nsd}	8	0.8641^{nsd}
SLA	1	0.0003***	2	0.1272^{nsd}	4	<0.0001***	4	0.0001***	2	0.8991^{nsd}	8	0.2791^{nsd}	8	0.1891^{nsd}
Leaf nitrogen	1	0.1848^{nsd}	2	0.099^{nsd}	3	<0.0001***	3	0.4876^{nsd}	2	0.2664^{nsd}	6	0.8264^{nsd}	6	0.6348^{nsd}
Aboveground biomass	1	<0.0001***	2	0.8535^{nsd}	2	0.1718^{nsd}	2	0.0001***	2	0.0104*	4	0.7651^{nsd}	4	0.7826^{nsd}
Leaf chlorophyll *a*	1	0.3330^{nsd}	2	0.4040^{nsd}					2	0.4000^{nsd}				
B														
CV of plant height 2009	1	<0.0010***	2	0.0799^{nsd}	5	0.1956^{nsd}	5	0.0234*	2	0.2954^{nsd}	10	0.6209^{nsd}	10	0.4732^{nsd}
CV of leaf dry weight	1	0.0110*	2	0.7293^{nsd}	4	0.0844^{nsd}	4	0.5310^{nsd}	2	0.7379^{nsd}	8	0.2522^{nsd}	8	0.9358^{nsd}
CV of leaf area	1	0.0147*	2	0.7869^{nsd}	4	0.7864^{nsd}	4	0.8709^{nsd}	2	0.6267^{nsd}	8	0.4241^{nsd}	8	0.8936^{nsd}
CV of SLA	1	0.3843^{nsd}	2	0.7909^{nsd}	4	0.0024**	4	0.7925^{nsd}	2	0.5822^{nsd}	8	0.9935^{nsd}	8	0.3480^{nsd}

*P<0.05; **P<0.01; ***P<0.001; nsd no significant difference

Leaf chlorophyll *a* was higher in the nutrient-poor substrate with more legume cover than in the nutrient-rich substrate (Table 3A, significant substrate effect and legume effect, Supplementary Table 2). As in the mesocosms, leaf nitrogen in the Habitat Garden was similar between substrates (Figure 4C; Table 3A) even though legume cover was higher in the nutrient-poor substrate and absolute values were higher in the low fertility substrate than in the more fertile one.

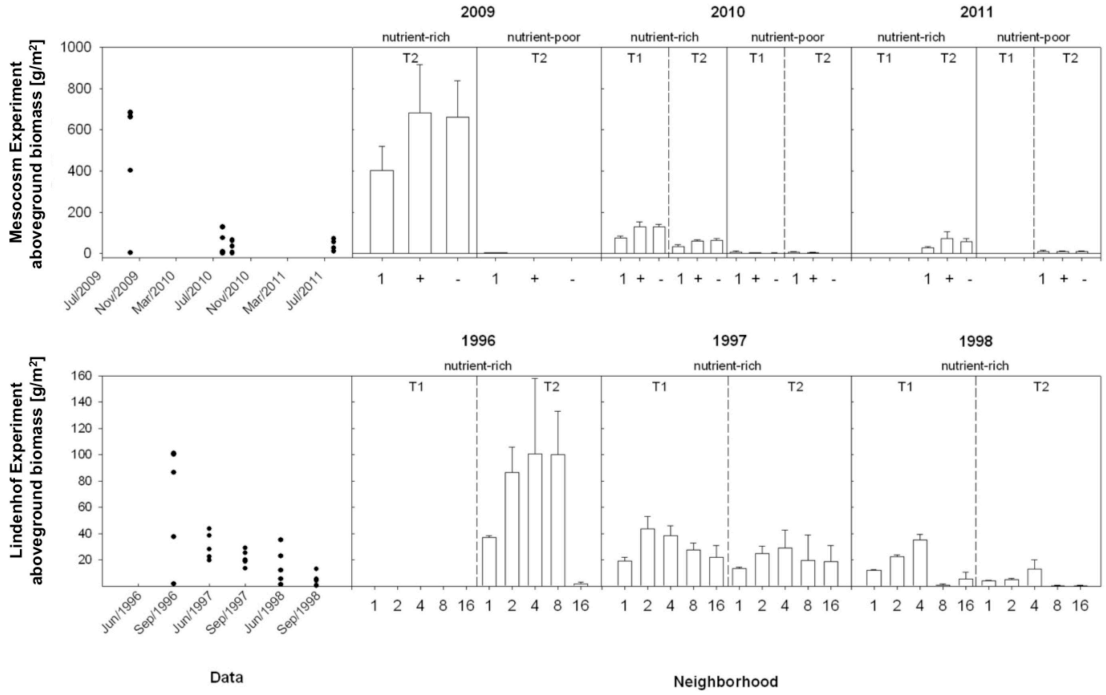

Figure 3. Effect of substrate and neighborhood on aboveground biomass of *P. lanceolata* over time **in the Mesocosm Experiment** and in the **Lindenhof Experiment** under field conditions (when the experiment was weeded). Each first panel shows the spread of the data over time independent of substrate and neighborhood. Next panels show aboveground biomass from 2009 to 2011 in the mesocosms separated by substrate type (nutrient-poor and –rich) and neighborhood (labels on x-axis: 1 = monoculture, + = with a legume neighbor, - = with a non-legume forb neighbor) and from 1996-1998 (during weeding) under field conditions at Lindenhof (where the soil was relatively nutrient-rich) and separated in neighborhoods (labels on x-axis: 1 (monoculture), 2, 4 (including one legume species), 8 (including one legume species) or 16 (including 4 legume species)). T1 and T2 indicated the two measurements points in each year (Mesocosm Experiment: August/September in 2010, July/August in 2011, Lindenhof Experiment: June/September). Values are means (+ standard error of the mean, for significant differences see Table 2 and 4).

Neighborhood and time effects on mean trait values

In the Mesocosm Experiment, despite mean plant height not being as affected by neighborhood as by substrate, in both growing seasons (in 2010 after the first biomass cutting event and 2011) height was significantly affected by neighborhood (Figure 1, Table 2A significant neighborhood effect). In the nutrient-rich substrate *P. lanceolata* was more productive (higher aboveground biomass) growing with another species than growing alone whereas in the nutrient-poor substrate this was not the case (Figure 3, Table 2A significant substrate X neighborhood interaction effect). In mesocosms leaf dry weight, leaf area, SLA and leaf nitrogen were unaffected by neighborhood (Table 2A no neighborhood effect). In 2011 leaf chlorophyll *a* was not different between substrate and neighborhood.

Table 3. Habitat Garden Experiment: Results of Repeated Measures ANOVA testing the effect of substrate (low and high nutrients) or neighborhood (lower or higher diversity plots, S2 vs. S7) as well as time (year) and their interactions on **(A)** mean trait values of *P. lanceolata* and legume cover or **(B)** coefficient of variation (CV) of traits of *P. lanceolata* (trait variability) under field conditions. Substrate or neighborhood effects were tested over the whole time span, whereas Year effects describe how the effect of the substrate factor or the neighborhood factor changed over time. Each line represents a single analysis. The response variable of leaf nitrogen was tested using one-way ANOVA (since measured at only one time point).

Response variable	Factors					
	Substrate		Year		Year X substrate	
A	d.f.	P	d.f.	P	d.f.	P
Leaf dry weight	1	0.5280^{nsd}	1	0.0908^{nsd}	1	0.9517^{nsd}
Leaf area	1	0.3970^{nsd}	1	0.0017**	1	0.0929^{nsd}
SLA	1	0.4086^{nsd}	1	0.0972^{nsd}	1	0.3609^{nsd}
Leaf chlorophyll *a*	1	0.0027**	1	0.6332^{nsd}	1	0.6233^{nsd}
Legume cover	1	0.0005***	1	0.3306^{nsd}	1	0.2225^{nsd}
Leaf nitrogen	1	0.5450^{nsd}				
B						
CV of leaf dry weight	1	0.4371^{nsd}	1	0.2978^{nsd}	1	0.7300^{nsd}
CV of leaf area	1	0.7400^{nsd}	1	0.8042^{nsd}	1	0.3446^{nsd}
CV of SLA	1	0.0568^{nsd}	1	0.0434*	1	0.2168^{nsd}
	Neighborhood		Year		Year X neighborhood	
A	d.f.	P	d.f.	P	d.f.	P
Leaf dry weight	1	0.8318^{nsd}	1	0.3254^{nsd}	1	0.2599^{nsd}
Leaf area	1	0.6600^{nsd}	1	0.1078^{nsd}	1	0.3821^{nsd}
SLA	1	0.4230^{nsd}	1	0.1963^{nsd}	1	0.9222^{nsd}
Leaf chlorophyll *a*	1	0.0136*	1	0.0776^{nsd}	1	0.0467*
Legume cover	1	0.0019**	1	0.7489^{nsd}	1	0.7320^{nsd}
Leaf nitrogen	1	0.0127*				
B						
CV of leaf dry weight	1	0.5816^{nsd}	1	0.8820^{nsd}	1	0.6366^{nsd}
CV of leaf area	1	0.3717^{nsd}	1	0.4465^{nsd}	1	0.8916^{nsd}
CV of SLA	1	0.9058^{nsd}	1	0.1204^{nsd}	1	0.9537^{nsd}

*P<0.05; **P<0.01; ***P<0.001; nsd no significant difference

In the Habitat Garden Experiment higher leaf nitrogen and chlorophyll *a* was found in the higher diversity plots (S7) with higher legume cover than in the lower diversity plots (S2) (Figure 4C, Table 3A, significant neighborhood effect), and this effect left a stronger signal in the plants than background soil fertility treatment (initial substrate, Table 3A no significant

substrate effect for leaf nitrogen, but significant neighborhood effect, higher chlorophyll *a* values in nutrient-poor substrate (also higher legume cover) than nutrient-rich substrate). Legume cover tended to play a role in mean trait differences of leaf chlorophyll *a* and leaf nitrogen in the Habitat Garden Experiment (Table 3, significant legume cover effect together with significant chlorophyll *a* and nitrogen effect).

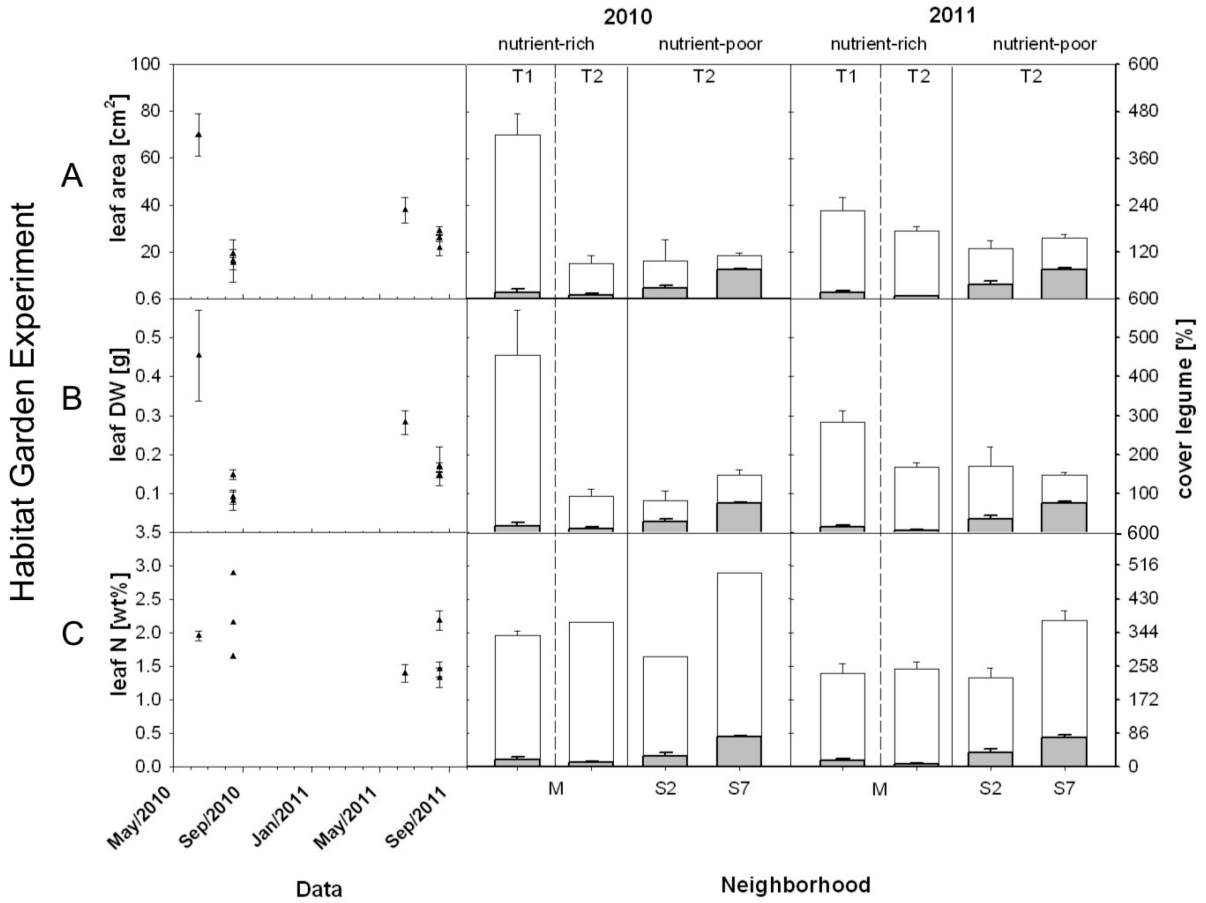

Figure 4. Habitat Garden Experiment: Effect of substrate and neighborhood on **(A)** leaf area, **(B)** leaf DW and **(C)** leaf nitrogen of *P. lanceolata* over time in the Habitat Garden Experiment under field conditions. Each first panel shows the spread of the data over time independent of substrate or neighborhood. Next panels show the time periods from 2010 to 2011 separated by substrate type (nutrient-poor (S) and –rich (M)) and neighborhood (S2 and S7 only in the nutrient-poor substrate). S2 = 2 grass species and 25 forbs (one of which was a legume) and S7 = 7 grass species and 32 forb species (four of which were legumes. T1 and T2 indicated the two measurements points (June and August) in each year. Values are means (+ standard error of the mean, for significant differences see Table 3). Grey bars represent the legume cover.

In the Lindenhof Experiment, aboveground biomass of *P. lanceolata* and legume cover were affected by neighborhood and year in the time period from 1996-1998 where plots were weeded as part of the Biodepth experiment (Figure 3, Table 4 weeding with a significant neighborhood effect, year and interaction effect). Aboveground biomass of *P. lanceolata* decreased over time as in the mesocosms. *P. lanceolata* was more productive with neighboring species than when growing in monocultures as in the mesocosms (although this was not the case for the 16 species composition in 1996 and for the 8 and 16 species composition in 1998; Figure 3). Aboveground biomass of *P. lanceolata* and legume cover in 2009-2011 where free succession was allowed was not affected by starting sown diversity (neighborhood) or time (Table 4 free succession No.1). When sown diversity wasn´t taken into account (in the 2009-2011 period where succession was allowed), aboveground biomass

of *P. lanceolata* was affected by time (Figure 5, Table 4 free succession No.2). *P. lanceolata* had rather stable populations (aboveground biomass) when comparing similar months of the year (June or September) and it did not respond much to legume cover in the plots but over all three years aboveground biomass of *P. lanceolata* was higher in the second vegetation peak after mowing than in the first vegetation peak (comparing June to September), but this effect got smaller over time (Figure 5, significant t-test in 2009 p = 0.046*).

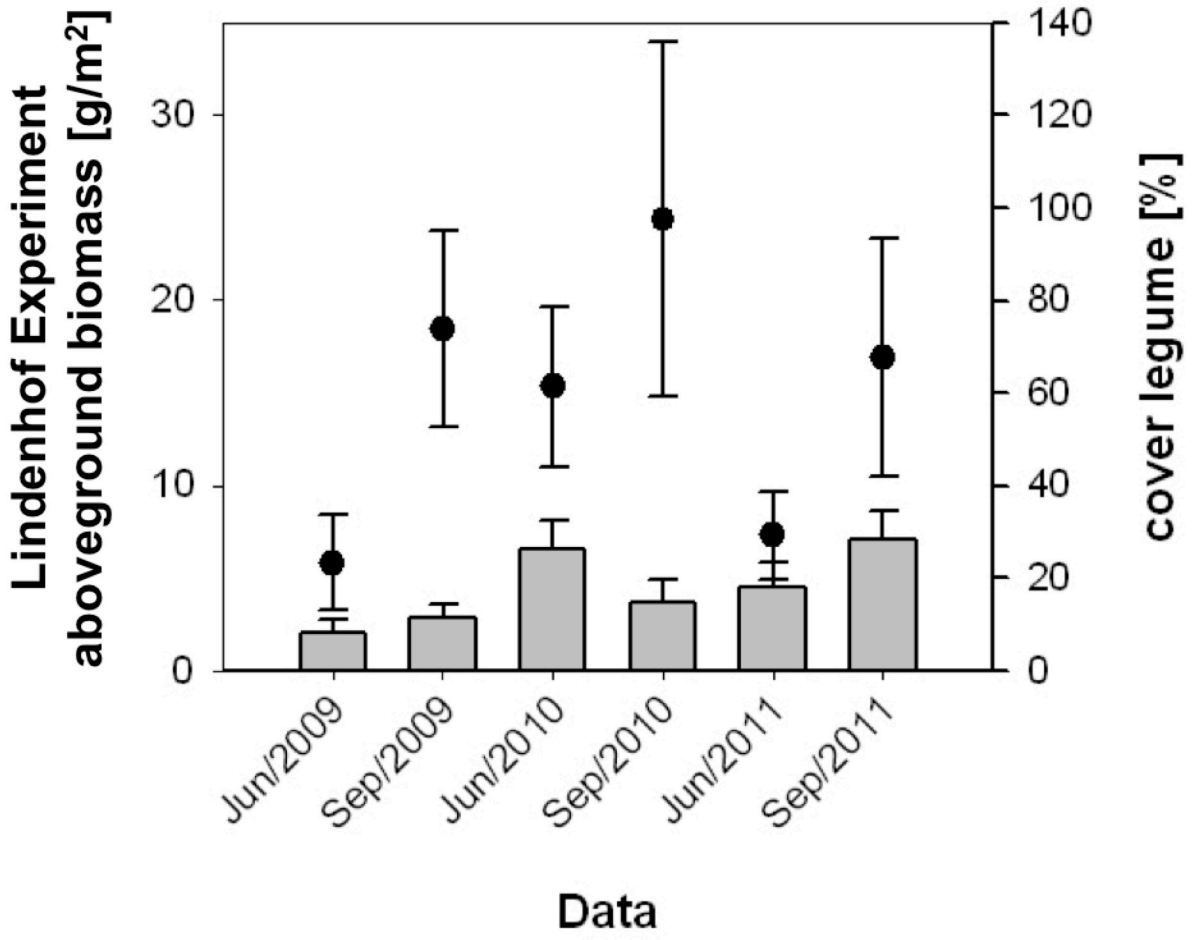

Figure 5. Lindenhof Experiment: Variation of aboveground biomass of *P. lanceolata* over time from 2009-2011 (ignoring any diversity effects e.g. data pooled) in the Lindenhof Experiment under field conditions (during the period where succession was allowed). Black dots show biomass data, grey bars represent legume cover. Values are means (± standard error of the mean, for significant differences see Table 4).

Table 4. Lindenhof Experiment: Results of Repeated Measures ANOVA on Lindenhof Experiment data testing the effect of neighborhood (*P. lanceolata* in monoculture and growing in 2, 4, 8, 16 species-plots), time (year) and their interactions on aboveground biomass and legume cover during weeding time as part of the Bayreuth BIODEPTH experiments (Hector et al. 1999) and later when plots were left for free succession. Neighborhood effects show results of testing over the whole time span, whereas year effects describe how the effect of the neighborhood factor changed over time. Each line represents a single analysis. For free succession No. 2 the test was also performed only for time without the factor of starting neighborhood/diversity from the beginning of the experiment.

Response variable		Factors					
		Neighborhood		Year		Year X neighborhood	
		d.f.	P	d.f.	P	d.f.	P
Weeding	Aboveground biomass	4	0.0307*	4	<0.0001***	16	0.0445*
	Legume cover	4	0.0008**	2	0.0028**	8	0.0001***
Free succession	1. Aboveground biomass	4	0.4156^{nsd}	5	0.1192^{nsd}	20	0.5216^{nsd}
	Legume cover	4	0.1912^{nsd}	5	0.1139^{nsd}	20	0.5285^{nsd}
	2. Aboveground biomass			5	<0.0001***		
	Legume cover			5	0.0565^{nsd}		

*P<0.05; **P<0.01; ***P<0.001; nsd no significant difference

Trait Variability (coefficient of variation)

Does the trait variability of P. lanceolata (coefficient of variation; CV), differ within and between experiments under the same tested conditions?

Trait variability in the Mesocosm and the Habitat Garden Experiment in detail for each replicate (calculated one CV value per replicate for leaf dry weight, leaf area, SLA and height)

Soil fertility and time effects on trait variability

Soil fertility had only a significant effect on the CV of three of the four in detail-tested traits (leaf dry weight, leaf area and height, no effect on SLA) in mesocosms. We found significantly higher trait variability in low fertility substrates and much less variability in the more fertile soils in mesocosms (Table 2B (significant substrate effect for leaf dry weight, leaf area and height) and Table 5 (higher absolute CV values)) as well a trend for this pattern in the Habitat Garden Experiment (Table 3B (no significant influence but higher absolute CV values in Table 5)). Also time had less influence on the tested traits than for the mean trait values described above. Over time in mesocosms the variability of plant height in the nutrient-rich substrate was more stable than in the nutrient-poor substrate where the variability increased over time (Table 2B, interaction Year x substrate effect). The variability of SLA decreased over time for the Mesocosm and Habitat Garden Experiment (Table 2B, and 3B (substrate), significant year effect).

Table 5. Coefficient of variation (CV) of traits of *P. lanceolata* (trait variability) across a range of conditions in **(A)** Mesocosm Experiment **(B)** Habitat Garden Experiment and **(C)** a comparison of the CV of *P. lanceolata* traits under mesocosm and field conditions (Habitat Garden). **(A)** showed trait variability (CV) of *P. lanceolata* across different substrates (nutrient-poor and nutrient-rich) and different neighborhoods (monoculture, growing with a legume species, growing with a non-legume forb species, and a mean value for growing with another species (legume and non-legume forb species (named "2 species"))), but independently of time in the Mesocosm Experiment. **(B) s**howed trait variability (CV) of *P. lanceolata* growing *on different substrates* (nutrient-poor and nutrient-rich) in the Habitat Garden Experiment. For the nutrient-poor substrate we also assessed the trait variability (CV) when growing in *different neighborhoods* (lower versus higher diversity plots; S2 vs. S7), but both independently of time. Data used are therefore pooled mean CV values over time, with time being the entire length of the particular measurement period in an experiment. **(C)** comparison of trait variability between the Mesocosm and the Habitat Garden Experiment, depending on substrate (nutrient-poor and nutrient-rich) in the growing seasons 2010 and 2011, but independently of neighborhood or species composition (values used are therefore pooled mean CV values across neighborhoods).

A			
Neighborhood	Traits	Nutrient-rich substrate	Nutrient-poor substrate
Mono	Leaf dry weight	0.310	0.451
Legume		0.331	0.624
Non-legume forb		0.318	0.542
2 species		0.325	0.583
Mono	Leaf area	0.343	0.409
Legume		0.298	0.486
Non-legume forb		0.278	0.448
2 species		0.288	0.467
Mono	SLA	0.255	0.256
Legume		0.260	0.341
Non-legume forb		0.266	0.333

2 species		0.263	0.337
Mono	Plant height	0.142	0.262
Legume		0.130	0.363
Non-legume forb		0.101	0.280
2 species		0.116	0.322

B

	Traits	Nutrient-rich substrate	Nutrient-poor substrate		
			S2 and S7	S2	S7
	Leaf dry weight	0.406	0.471	0.514	0.428
	Leaf area	0.340	0.401	0.481	0.321
	SLA	0.233	0.427	0.439	0.415

C

Time	Traits	Nutrient-rich substrate		Nutrient-poor substrate	
		Habitat Garden	Mesocosm	Habitat Garden	Mesocosm
2010	Leaf dry weight	0.415	0.335	0.454	0.667
	Leaf area	0.284	0.267	0.450	0.392
	SLA	0.288	0.146	0.574	0.326
2011	Leaf dry weight	0.396	0.373	0.488	0.483
	Leaf area	0.396	0.354	0.352	0.433
	SLA	0.178	0.177	0.279	0.205

Neighborhood effects on trait variability

Significant neighborhood or interaction effects did not occur in both experiments (Table 2B and 3B2), although in nutrient-poor substrate the absolute CV trait values were higher (less stable) in two species combination than in monoculture in the mesocosms, in the nutrient-rich substrate it depended on the trait you looked at, whereas in the nutrient-poor substrate of the Habitat Garden Experiment the lower diversity plots (S2) had higher absolute CV trait values than the higher diversity plots (S7) with more legume cover (Table 5).

Mesocosm Experiment versus Habitat Garden Experiment

Trait variability was more different and the stability lower (significant differences in CV values) under the tested conditions within the Mesocosm Experiment but was relatively stable (not changing significantly) under field settings in the Habitat Garden Experiment (Table 2B, 3A2/B2). When comparing the absolute CV values within one tested condition the variability was smaller in the nutrient-rich substrate for the mesocosms in the tested traits in both years in comparison to the Habitat Garden Experiment, whereas in the nutrient-poor substrate it depends on the trait and the year you looked at (Table 5).

Trait variability in the Mesocosm, Habitat Garden and Lindenhof Experiments at population level (calculated one CV value per treatment/across all replicates for each measured traits)

When looking at the absolute values of the population trait variability, we found same patterns/influences for substrate and neighborhood in the Mesocosm, the Habitat Garden Experiment and in their comparison (see Supplementary Table 3 and 4 for more Information) as we also found in the detailed analysis of the trait variability described above.

In the Lindenhof Experiment the population variability of aboveground biomass was higher with more species neighbors than in a monoculture (CV value increased with increasing species, Supplementary Table 3C and Table 4B weeding time). This pattern was also found

for aboveground biomass in the mesocosms for the nutrient-rich substrate (Supplementary Table 3A). Comparing population variability of aboveground biomass between weeding and free succession in the Lindenhof Experiment showed that the CV value of aboveground biomass was higher in the time of free succession and was unregulated by species diversity in this time period (Supplementary Table 3C and Table 4B). Previous species combination/diversity of the plots had no influence on the population variability of aboveground biomass during the period where free succession was allowed (Supplementary Table 3C)**.** Population variability of aboveground biomass of *P. lanceolata* was higher in the Mesocosm than in the Lindenhof Experiment (Supplementary Table 4B).

Discussion

Variation of trait means across a range of abiotic and biotic conditions

The factors substrate and time had stronger effects on the variation in traits of *P. lanceolata* under more controlled mesocosms than in field settings in the Habitat Garden Experiment (Table 2A and 3A). Although the two substrate-types tested were similar in terms of soil fertility in both experiments, one difference was that the nutrient-rich substrate in the mesocosms had a higher total C amount in contrast to the nutrient-rich substrate in the Habitat-Garden Experiment. Furthermore, site conditions such as soil temperature or water availability could differ more between the two substrates in the mesocosms than they vary the field, which may lead to higher differences in trait variation.

However, in the Habitat Garden Experiment neighborhood (especially legume cover) had a stronger effect on trait differences than in mesocosms (Table 2A and 3A). So the Mesocosm Experiment under more controlled conditions confirms our first hypothesis, that soil fertility will have stronger effect on trait variation than neighborhood and time. Under field condition, however, this could not be confirmed.

On the one hand, we found that plants were able to change their phenotype in response to environmental change. Indeed, it is often assumed that phenotypic plasticity has evolved again and again as an adaptation to environmental heterogeneity. On the other hand, many phenotypic responses to stressful environments may just be the consequence of passive reductions in growth due to resource limitation. Van Kleunen and Fischer (2005) stated that active and passive plastic responses of plants may act at the same time. Thus, our observed phenotypic responses to the environment may be the net result of both passive responses as a consequence of resource limitation and active responses as a consequence of changes in allocation. This is a hypothesis however, and needs further testing. The fact that plasticity observed in nature is often lower than that expected suggests the existence of costs and limits to phenotypic plasticity.

Many internal and ecological factors can influence the capacity of plants to respond to a given environmental factor. Different selective pressures and limitations may act upon the maximization of plasticity. In our case, lower soil nutrient availability seems to have led to high passive plasticity and therefore to significant differences in trait means between the substrate types. In contrast, in the field, the multiple factors simultaneously acting on traits seem to have led to a more stable observed variation (plasticity). Passive and active plasticity may therefore have cancelled each other out, but there was also an indication that positive legume effects could increase variation in some traits in the Habitat Garden Experiment (see Table 3, Figure 4). Gubsch et al. (2011) found for grass species growing in an experimental grassland that traits related to N acquisition and use (e.g. leaf nitrogen concentrations) did not change with plant species richness but were strongly influenced by legume presence.

Over time, the strong initial effect of substrate type on trait means in the mesocosms leveled out, and this was because trait means were more variable and decreased over time in nutrient-rich substrates compared to more stable traits (less increase) over time in nutrient-poor substrates (see Figure 1 and 2). This shows that *P. lanceolata* can adapt to the more extreme substrate and is able to be more productive (plant height, aboveground biomass, leaf area, leaf dry weight) after a while in that environment than at the beginning, even though total soil N stayed stable over time. In this case plasticity seemed to be advantageous when the response could occur late in development and the plants could respond to an environmental factor faster than the level of the factor changes (N stayed stable). Alpert and Simms (2002) have described this phenomenon in their study about the relative advantages of plasticity. Furthermore Lambers et al. (2008) found that soil properties that are relevant for nutrient acquisition (especially nitrogen and phosphorus) change strongly as soils develop over time, but plants are often equipped with a wide array of traits that are regarded as adaptations for overcoming these limitations (e.g. mechanisms of resorption, internal recycling, and allocation and use of N and P in growth).

In the mesocosms physiological traits (chlorophyll *a*, leaf nitrogen) were less affected by abiotic and biotic conditions than other traits more directly related to growth (compared to in field settings in the Habitat Garden Experiment) (Table 2A and 3A). Overall, neighborhood (especially higher legume cover) and substrate type affected leaf chlorophyll *a* and leaf nitrogen in the field (Habitat Garden Experiment) (Table 3A) but not in the Mesocosm Experiment (Table 2A). Higher soil nitrogen should lead to higher chlorophyll *a* and nitrogen content in leaves (Minotta and Pinzauti, 1996; Ordoñez et al., 2009), and in our study more legume cover in the nutrient-poor substrate seemed to positively influence leaf nitrogen indirectly via its effect on soil N availability. It is intriguing that our study has found evidence that the influence of legumes may be stronger under field conditions rather than under more controlled conditions. This effect needs further study, and this outcome may have important implications for scaling up from controlled experiments to field and landscape scales (Poorter et al., 2012 and 2016).

Lambers et al. (2012) also showed that species on poor P soils as in Western Australia can photosynthesize at similar rates despite extremely low P in soils in this region. Plants can be highly efficient in their N use across a wide range of N availabilities in soils (Lambers et al., 2008). They seem to store excess nitrogen (often as Rubisco; Warren et al., 2000) and are able to perform equally well physiologically even under low or extremely low nutrient availability (Temperton et al., 2003 a and b). Our study confirms this statement, in that leaf nitrogen was more similar between the substrate fertility levels and even converged over time (sometimes even with higher leaf nitrogen values in the low nutrient substrate with more legume cover).

In the nutrient-rich substrate in the Mesocosm Experiment as well as in the Lindenhof Experiment, *P. lanceolata* was more productive when growing in polyculture compared to growing in monoculture (Figure 3). However, this positive interaction was not found for the nutrient-poor substrate. Resource limitation obviously modifies the biotic interactions between species. As an adaptation to the land use regime in hay meadows, *P. lanceolata* was more productive or performed faster reproduction after mowing and cutting (Figure 5). Taken together these results suggest a strong adaptive capacity: *P. lanceolata* is able to grow fast and is able to react rapidly to competition under more nutrient-rich conditions. Lepik et al. (2005) also show that high shoot plasticity favors plant coexistence in herbaceous communities.

Variability and stability of traits (coefficient of variation) across a range of abiotic and biotic conditions

Overall, soil fertility had a significant effect on the trait variability only in the Mesocosm Experiment, whereas significant neighborhood or interaction effects did not occur in the Mesocosm as well as in the Habitat Garden Experiment (Table 2B and 3B). Time also did not have such strong influence on trait variability, as soil fertility. Time influenced the variability of SLA. This also confirms our first hypothesis, that soil fertility will have stronger effects on trait variation. This could be also an indication for a high passive response of plasticity and a high and differentiated selective pressure on each individual. Larger intraspecific functional variability may enable species to adapt to a wider range of interaction and abiotic conditions and therefore have greater niche breadth (Sides et al., 2014). This would certainly fit the niche breadth of *P. lanceolata*, a grassland species found both in mesotrophic and dry acidic grasslands. As such we hypothesize that the strong capacity of *P. lanceolata* to adapt plastically to its environment as found in our study, may be quite different for species with a narrower niche breadth, that are more specialized to specific abiotic environments.

In our study trait variability was higher in the low fertility substrates than in the more fertile substrate in the mesocosms, (whereas in the Habitat Garden Experiment we only found a trend for this pattern as well as for the population trait variability) (Table 2B, 3B, 5A and 5B). However theory predicts that plastic responses to abiotic factors are reduced in less favorable and more stressful sites and that extreme levels in a given abiotic factor can negatively influence plastic responses to another factor (Valladares et al., 2007). Here, phenotypic plasticity is considered advantageous when (mean) resource availability is higher, when a response can occur late in development and when a response is reversible. Alpert and Simms (2002) considered that selection is likely to favor plasticity when "an environmental factor varies on the same spatial scale as the plant response unit, when the plant can respond faster than the level of the factor changes and when environmental variation is highly but not completely predictable". They found however, that the available evidence does not support their hypothesis that high mean resource availability necessarily favors plasticity. Therefore plasticity could be advantageous under some conditions and disadvantageous or not advantageous under other. This, in turn, leads to differences in phenotypic plasticity. Lemke et al. (2012) also show that in most of species tested in their study (five forest herbs) both vegetative and reproductive traits were more variable in sites with higher soil nutrient content and higher light availability.

There was a trend that the stability of traits (CV; in the detailed analysis and on population level) was lower in *P. lanceolata* when growing in more diverse communities than in monocultures, at least in the nutrient-poor substrate in the mesocosms (Table 5A and Supplementary Table 3A). *P. lanceolata* tended to express strong variability in traits when experiencing interspecific competition, whereas trait convergence occurred when experiencing predominantly intraspecific competition in our study. This agrees with other studies in which competition leads to higher variability (Callaway et al., 2003; Lichtstein et al., 2007). The passive response due to low nutrient availability is similar whereas interspecific competition led to higher pressure on changes in phenotypic variation. This could be an indication of adaptive plasticity.

But in contrast to the Mesocosm Experiment it seems that there was a higher trait variability (CV in detail and on population level) in the lower sown diversity plots (S2) than in the more sown diverse plots of the nutrient-poor substrate (S7) in the Habitat Garden Experiment (Table 5B and Supplementary Table 3B). In this case the more diverse plots (S7) had also more legume cover which could lead to higher stability of traits, because of the positive

legume effect on available N. The population variability of aboveground biomass was higher in more diverse communities than in monocultures in the Lindenhof Experiment as well as in the mesocosms (Supplementary Table 3A and 3C). The population variability of aboveground biomass during the time after weeding in the Lindenhof Experiment (free succession) was higher than during weeding (stable diversity treatment). This fits with the fact that in diverse communities different individuals of the same species are generally more likely to interact with individuals of different species than in less diverse communities.

Trait variation (mean trait differences and trait variability) and how they varied in mesocosms versus field conditions.

Trait convergence can be seen as a form of plant adaptation to tradeoffs and an ability to grow in a wide range of environments (Diaz et al., 2016). Under the tested conditions in our study we found more evidence for trait convergence in the field than under more controlled conditions (less significant differences in mean trait values as well in trait variability/CV values). This thus confirms our second hypothesis that trait variation in *P. lanceolata* will be lower under field conditions compared to when growing in mesocosms under the tested conditions. The abiotic and biotic conditions *P. lanceolata* plants experienced in mesocosms seemed to have more influence on the variation of traits than in plants growing in the field. We suggest that the larger range of influences on fitness in the field (heterogeneity of resource availability, competition, herbivory, weather) may select for a more conservative phenotypic plasticity. In detail, however, it does also depend on which factor and which trait you look at. Lemke et al. (2012) also found that differences in trait variability compared between different regions, depends on which trait and which species you look at. But comparing the absolute CV values under similar soil conditions however, the variability was smaller in the nutrient-rich substrate for the mesocosms compared to the Habitat Garden Experiment (Table 5C). In contrast, in the nutrient-poor substrate it depended on the trait and the year considered. This shows that under different conditions in the field *P. lanceolata* showed less differences in trait variation than in the mesocosms, but had under some same conditions a higher intraspecific variability (in absolute values) than in the mesocosms.

In heterogeneous environments plant species populations show greater adaptive plasticity than populations from homogeneous sites (see Review: Matesanz et al., 2010). Under similar soil treatments, we found quite specific responses in trait variation for field and also for controlled conditions in the mesocosms. This implies caution when interpolating between lab and field studies or across environmental gradients, since translation of knowledge is not necessarily straightforward (Poorter et al., 2016). De Boeck et al. (2015) also reviewed that "in assembled systems, many aspects of complexity are not included in order to safeguard high internal validity and that Experiments could gain in realism if aspects such as more natural demography, colonization rates, or intraspecific variation were introduced, albeit at the cost of internal validity". The latter advocate taking the approach we follow in this study, which is to compare plant performance across similar abiotic/soil conditions and in the lab experiments to try to simulate conditions found in the field.

Conclusion

We found that *P. lanceolata* exhibits a range of trait plasticity under experimental conditions with different degrees of control and naturalness. Traits varied more strongly under the more-controlled mesocosm conditions than in the field. This applied both to the means and the variability/stability of the traits. Soil fertility and especially non-optimal abiotic conditions led to higher plasticity under the more-controlled mesocosm conditions. The multivariate factors, which act simultaneously on *P. lanceolata* traits in both field habitats, led to a more conservative trait plasticity and more trait convergence, as well as a stronger effect of legume

neighborhood. Both differences in genetic variation and environmental variation could be responsible for the observed patterns and the differences in phenotypic expression of traits.

We predict that species with a wide adaptive plasticity such as *P. lanceolata* will be more likely to survive under novel environmental conditions driven by climate change and other global change drivers than those with lesser adaptive plasticity.

Author Contributions
VT designed and established the Habitat Garden Experiment, CB the Lindenhof Experiment and CP and VT the Mesocosm Experiment. CP collected the data of the Habitat Garden and Mesocosm Experiment, CB of the Lindenhof Experiment. CP analyzed the data. CP and VT led the writing of the Manuscript. CB reviewed the manuscript.

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Supplementary Material
The Supplementary Material related to this article can be found online at XXXX.

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Supplementary Material

Supplementary Table 1. Mesocosm Experiment: The development of additional traits of *P. lanceolata* and soil chemistry data in relation to substrate (nutrient-poor and nutrient-rich) and neighborhood (monoculture, growing with a legume species or growing with a non-legume forb species) over time (2009-2011). Values are means (± standard error of the mean).- indicates no data available.

Time	Substrate	Neighborhood	Chlorophyll *a* µg/cm^2		SLA cm^2/g		ETR_{max} µmol/e m^2 s		F_v/F_m		Soil %C		Soil %N	
			MV	S.E.	MV	S.E.	MV	S.E.	MV	S.E.	MV	S.E.	MV	S.E.
10/2009	Nutrient-rich	Monoculture	24.392	3.169	133.852	16.976	81.970	-	0.734	-	Start 3.56	-	Start 0.127	-
		Legume	25.107	1.711	185.781	19.742	88.060	-	0.790	-		-		-
		Non-legume forb	28.183	2.533	200.878	39.139	86.520	-	0.760	-		-		-
10/2009	Nutrient-poor	Monoculture	6.765	-	285.124	43.248	-	-	-	-	Start 0.57	-	Start 0.02	-
		Legume	64.730	-	188.622	54.580	-	-	-	-		-		-
		Non-legume forb	14.185	-	274.544	116.220	-	-	-	-		-		-
08/2010	Nutrient-rich	Monoculture	15.250	-	87.528	10.822	234.033	14.296	0.686	0.004	6.950	-	0.200	-
		Legume	16.600	-	90.701	4.337	173.783	36.253	0.669	0.025	8.390	-	0.208	-
		Non-legume forb	26.460	-	85.785	14.708	151.567	17.804	0.711	0.012	9.650	-	0.246	-
09/2010	Nutrient-rich	Monoculture	14.320	-	129.160	5.277	-	-	-	-	5.340	-	0.139	-
		Legume	17.890	-	170.658	31.978	-	-	-	-	6.970	-	0.192	-
		Non-legume forb	12.660	-	122.449	15.648	-	-	-	-	5.570	-	0.147	-
08/2010	Nutrient-poor	Monoculture	15.470	-	182.595	26.830	189.500	5.247	0.647	0.031	1.050	-	0.026	-
		Legume	16.377	-	218.758	21.728	171.633	11.464	0.651	0.033	1.500	-	0.045	-
		Non-legume forb	11.305	-	181.739	15.149	165.300	6.203	0.644	0.033	1.110	-	0.027	-
09/2010	Nutrient-poor	Monoculture	14.350	-	186.260	12.766	-	-	-	-	1.100	-	0.029	-
		Legume	16.500	-	223.459	11.895	-	-	-	-	1.010	-	0.029	-
		Non-legume forb	18.087	-	174.940	22.489	-	-	-	-	1.250	-	0.029	-
07/2011	Nutrient-rich	Monoculture	21.150	-	116.278	6.712	198.800	39.015	0.710	0.006	6.310	-	0.174	-
		Legume	20.200	-	126.868	5.004	177.833	7.441	0.735	0.013	3.480	-	0.104	-
		Non-legume forb	15.770	-	139.317	8.860	196.267	13.986	0.731	0.014	7.090	-	0.187	-
08/2011	Nutrient-rich	Monoculture	20.717	0.668	189.560	3.803	-	-	-	-	5.160	-	0.141	-
		Legume	18.493	0.183	225.503	10.658	-	-	-	-	6.350	-	0.178	-
		Non-legume forb	20.553	1.135	209.763	21.619	-	-	-	-	4.700	-	0.118	-
07/2011	Nutrient-poor	Monoculture	13.730	-	98.903	6.961	228.133	11.009	0.689	0.049	0.885	-	0.021	-
		Legume	7.370	-	204.390	33.210	177.033	29.187	0.736	0.009	1.480	-	0.039	-
		Non-legume forb	20.890	-	174.609	10.197	209.800	49.214	0.670	0.061	1.520	-	0.035	-
08/2011	Nutrient-poor	Monoculture	21.303	3.146	178.531	39.458	-	-	-	-	0.777	-	0.022	-
		Legume	20.753	2.715	185.205	29.516	-	-	-	-	0.777	-	0.022	-
		Non-legume forb	19.447	4.374	212.881	46.858	-	-	-	-	1.030	-	0.026	-

Supplementary Table 2. Habitat Garden Experiment: The development of additional traits of *P. lanceolata* and soil chemistry data depending on substrate (nutrient-poor (S) and nutrient-rich (M)) and neighborhood (lower versus higher diversity plots; S2 vs. S7) over time (2010-2011). Values are means (± standard error of the mean). - indicated no available data.

Time	Substrate	Treatment	Chlorophyll *a* µg/cm^2		SLA cm^2/g		ETR_{max} µmol/e m^2 s		F_v/F_m		Soil %C		Soil %N	
			MV	S.E.	MV	S.E.	MV	S.E.	MV	S.E.	MV	S.E.	MV	S.E.
06/2010	Nutrient-rich	M	28.420	2.553	247.816	70.017	-	-	-	-	1.650	0.109	0.112	0.004
08/2010			20.382	0.921	179.753	17.201	236.542	13.956	0.692	0.012	1.647	0.056	0.114	0.003
08/2010	Nutrient-poor	S2	21.330	1.370	253.105	124.948	194.950	40.050	0.706	0.011	0.511	0.004	0.044	0.003
		S7	37.577	5.297	148.743	1.558	212.150	31.680	0.721	0.024	0.905	0.217	0.059	0.013
06/2011	Nutrient-rich	M	33.205	2.251	133.892	10.326	152.867	18.471	0.743	0.012	1.410	0.077	0.105	0.004
08/2011			22.093	1.353	179.070	10.979	227.050	15.905	0.731	0.005	1.292	0.041	0.095	0.003
08/2011	Nutrient-poor	S2	26.480	1.102	156.160	1.651	-	-	-	-	0.351	0.039	0.013	0.003
		S7	29.007	1.528	188.734	8.344	-	-	-	-	0.441	0.014	0.018	0.000

Supplementary Table 3. Coefficient of variation (CV) of all tested *P. lanceolata* traits across a range of conditions and experiments (**A-C**). (**A)** showed population trait variability (CV) of *P. lanceolata* across different substrates (nutrient-poor and nutrient-rich) and different neighborhoods (monoculture, growing with a legume species, growing with a non-legume forb species, and a mean value for growing with another species (legume and non-legume forb species (named "2 species"))), but independently of time in the Mesocosm Experiment. (**B) s**howed population trait variability (CV) of *P. lanceolata* growing *on different substrates* (nutrient-poor and nutrient-rich) in the Habitat Garden Experiment. For the nutrient-poor substrate we also assessed the population trait variability (CV) when growing in *different neighborhoods* (lower versus higher diversity plots; S2 vs. S7), but both independently of time. **(C)** showed population trait variability (CV) of *P. lanceolata* between weeded and non-weeded plots (free succession) as well as between different neighborhoods (*P. lanceolata* monoculture and *P. lanceolata* growing in 2, 4, 8, 16 species-plots), but independently of time in the Lindenhof Experiment. Data used are therefore pooled mean CV values over time, with time being the entire length of the particular measurement period in an experiment.

A			
Neighborhood	Traits	Mesocosm	
		Nutrient-rich substrate	Nutrient-poor substrate
Mono	Leaf dry weight	0.238	0.330
Legume		0.172	0.722
Non-legume forb		0.226	0.558
2 species		0.199	0.631
Mono	Leaf area	0.229	0.387
Legume		0.156	0.805
Non-legume forb		0.252	0.444
2 species		0.204	0.604
Mono	SLA	0.128	0.228
Legume		0.148	0.260
Non-legume forb		0.229	0.317
2 species		0.189	0.292
Mono	Leaf nitrogen	0.196	0.246
Legume		0.119	0.218
Non-legume forb		0.187	0.309
2 species		0.153	0.263
Mono	Leaf chlorophyll *a*	0.140	0.256
Legume		0.068	0.227
Non-legume forb		0.126	0.390
2 species		0.097	0.308
Mono	Plant height	0.104	0.252
Legume		0.087	0.470
Non-legume forb		0.120	0.199
2 species		0.104	0.335
Mono	Aboveground biomass	0.376	0.650
Legume		0.479	0.737
Non-legume forb		0.334	0.709
2 species		0.407	0.723

B					
	Traits	Habitat Garden			
		Nutrient-rich substrate	Nutrient-poor substrate		
			S2 und S7	S2	S7
	Leaf dry weight	0.305	0.285	0.458	0.112
	Leaf area	0.263	0.327	0.525	0.130
	SLA	0.253	0.203	0.358	0.047
	Leaf nitrogen	0.128	0.149	0.185	0.113
	Leaf chlorophyll *a*	0.117	0.125	0.081	0.168

C				
Neighborhood	Traits	Lindenhof		
		Weeded	Free succession	
Mono	Aboveground biomass	0.105	0.894	
2 species		0.261	1.063	
4 species		0.537	0.576	
8 species		0.989	1.001	
16 species		1.151	0.856	

Supplementary Table 4. Comparison of the coefficient of variation (CV, population trait variability see Table 5 for more details on the two different CV calculation methods) of all tested *P. lanceolata* traits under mesocosm and field conditions (**A)** comparison of population trait variability between the Mesocosm and the Habitat Garden Experiment, depending on substrate (nutrient-poor and nutrient-rich) in the growing seasons 2010 and 2011, but independently of neighborhood or species composition (values used are therefore pooled mean CV values across neighborhoods) **(B)** comparison of population trait variability of *P. lanceolata* growing in monoculture and with another neighboring species in mesocosms (Note: for the mesocosms the CV for 2 species are the pooled mean values of the CV of *P. lanceolata* growing with a legume and a non-legume forb species) and in the Lindenhof Experiment during the first three years of the establishment of the experiment (weeding) and in between 2009-2011 free succession.

A

Time		Traits	Nutrient-poor substrate		Nutrient-rich substrate	
			Habitat Garden	Mesocosm	Habitat Garden	Mesocosm
2010		Leaf dry weight	0.278	0.447	0.472	0.266
		Leaf area	0.468	0.374	0.494	0.207
		SLA	0.358	0.145	0.234	0.206
		Leaf nitrogen	-	0.164	-	0.16
		Leaf chlorophyll *a*	0.167	-	0.111	-
2011		Leaf dry weight	0.292	0.425	0.160	0.222
		Leaf area	0.187	0.670	0.163	0.227
		SLA	0.047	0.347	0.150	0.098
		Leaf nitrogen	0.149	0.541	0.181	0.121
		Leaf chlorophyll *a*	0.082	0.291	0.087	0.056

B

Time	Neighborhood	Traits	Nutrient-rich substrate		
			Mesocosm	Lindenhof weeding	Lindenhof free succession
1	Mono	Aboveground biomass	0.515	0.055	0.795
	2 species		0.526	0.322	1.129
2	Mono		0.223	0.205	0.420
	2 species		0.253	0.312	1.414
3	Mono		0.440	0.107	0.259
	2 species		0.180	0.320	0.704
4	Mono		-	0.087	1.414
	2 species		-	0.086	0.432
5	Mono		0.326	0.071	1.414
	2 species		0.667	0.263	1.414

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9 Declarations

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